THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

DISCUSSION MEETING

A DISCUSSION Meeting of the Society was held at 6.30 p.m. on Wednesday, December 7th, 1960, in the Main Chemistry Lecture Theatre, Imperial College of Science and Technology, Imperial Institute Road, London, S.W.7. The Chair was taken by the President, Mr. R. C. Chirnside, F.R.I.C.

The subject for discussion was "The Flask Combustion Technique," which was opened by W. Schöniger, Dr.ing. The following short contributions were presented and discussed: "Apparatus for Electrical Ignition," by J. Haslam, D.Sc., F.R.I.C., J. B. Hamilton and D. C. M. Squirrell, B.Sc., F.R.I.C.; "Determination of Halogens," by C. A. Johnson, B.Pharm., B.Sc., F.P.S., F.R.I.C.; "Determination of Boron," by Miss M. Corner, M.Sc., F.R.I.C.; "Determination of Phosphorus," by E. Q. Laws, B.Sc., F.R.I.C.; "Determination of Sulphur," by Miss J. P. Dixon, A.R.I.C.

NORTH OF ENGLAND SECTION AND MICROCHEMISTRY GROUP

A JOINT Meeting of the North of England Section and the Microchemistry Group of the Society with the North Wales Section of the Royal Institute of Chemistry was held at 7.15 p.m. on Friday, September 30th, 1960, at the University College of North Wales, Bangor, Caernarvonshire. The Chair was taken by the Chairman of the Microchemistry Group, Mr. F. Holmes, B.Sc., A.R.I.C.

The subject of the meeting was "Techniques and Scales of Analysis" and the speakers were Professor R. Belcher, Ph.D., D.Sc., F.R.I.C., F.Inst.F., and Professor C. L. Wilson, Ph.D., D.Sc., F.R.I.C., F.I.C.I. (see summaries below).

On the morning of Saturday, October 1st, 1960, there was a visit to the Explosive Works at Penrhyndeudraeth. At 8.45 p.m. on the same day there was a discussion meeting on "Techniques and Scales of Analysis," at which the Chair was once again taken by Mr. Holmes.

TECHNIQUES AND SCALES OF ANALYSIS

PROFESSOR BELCHER gave instances of the confusion that could arise with any of the usual definitions of scales of analysis, which must relate both to the starting material and to the material sought, as well as to the techniques used. He surveyed the work carried out at Birmingham on the ultra-micro determination, using 30- to 50-μg samples, of halogens, nitrogen, sulphur, carbon, phosphorus, arsenic and functional groups, and of molecular weight. He emphasised that in many instances the weight of sample taken was equivalent to the tolerable error in the corresponding conventional micro-determinations.

PROFESSOR WILSON surveyed the changes that had taken place in analysis during the last half-century and, in view of the present-day reliance on instrumental methods, wondered if the term "chemical analysis" was still appropriate. The question of

definition of scale and of technique could only be resolved by considering separately the individual processes of analysis: sampling, measurement of sample, preliminary treatment, separation and measurement of constituent. He gave examples of the variations now possible within these processes, and concluded that the analyst might nowadays regard himself not as a specialist in any scale or technique of analysis, but simply as a chemist.

NORTH OF ENGLAND SECTION

AN Ordinary Meeting of the Section was held on Friday, November 4th, 1960, at the Great Northern Hotel, Leeds, 1. The Chair was taken by the Chairman of the Section, Dr. J. R. Edisbury.

The following paper was presented and discussed: "The Analytical Chemistry of Phosphorus," by N. T. Wilkinson, F.R.I.C.

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held at 7.15 p.m. on Friday, October 28th, 1960, at the Royal Society of Edinburgh, 22 George Street, Edinburgh, 2. The Chair was taken by the Chairman of the Section, Mr. A. N. Harrow, A.H.-W.C., F.R.I.C.

The following papers were presented and discussed: "Some Aspects of the Analytical Chemistry of the Heteropolymolybdates," by R. A. Chalmers, B.Sc., Ph.D., and A. G. Sinclair, B.Sc.; "A Colorimetric Determination of Sulphite," by W. Moser, B.Sc., R.A. Chalmers, B.Sc., Ph.D., and A.G. Fogg; "Some Factors Affecting the Use of Amalgams as Reductants," by R. A. Chalmers, B.Sc., Ph.D., W. Moser, B.Sc., and D. A. Thompson, B.Sc.

WESTERN SECTION

A JOINT Meeting of the Western Section of the Society with the Mid-Southern Counties Section of the Royal Institute of Chemistry was held at 7.45 p.m. on Friday, October 21st, 1960, at the Red Lion Hotel, Salisbury. The Chair was taken by the Chairman of the Mid-Southern Counties Section, Mr. D. H. Bell.

The following paper was presented and discussed: "Some Observations on Analytical Chemistry," by J. Haslam. D.Sc., F.R.I.C.

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 7 p.m. on Thursday, October 27th, 1960, at the Nottingham and District Technical College, Burton Street, Nottingham. The Chair was taken by Mr. W. S. Stephenson, D.B.A., F.P.S., F.R.I.C.

The following paper was presented and discussed: "Paper Chromatography of Dyestuffs," by J. C. Brown.

A JOINT Meeting of the Midlands Section with the Birmingham and Midlands Section of the Royal Institute of Chemistry was held at 6.30 p.m. on Thursday, November 10th, 1960, at the College of Technology, Gosta Green, Birmingham. The Chair was taken by the Chairman of the Birmingham and Midlands Section, Mr. G. King, M.B.E., M.Sc.

The following paper was presented and discussed: "Analytical Problems in Forensic Toxicology," by F. L. Cann, B.Sc., A.R.I.C.

MICROCHEMISTRY GROUP

THE twenty-sixth London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, October 26th, 1960, at "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Vice-Chairman of the Group, Mr. C. Whalley, B.Sc., F.R.I.C.

A discussion on "Analytical Aspects of Corrosion" was opened by H. C. J. Saint, A.R.I.C.

PHYSICAL METHODS GROUP

A JOINT Meeting of the Physical Methods Group with the Thames Valley Section of the Royal Institute of Chemistry was held at 6.30 p.m. on Tuesday, October 25th, 1960, in the Inorganic Chemistry Lecture Theatre, The University, Oxford. The Chair was taken by the Chairman of the Physical Methods Group, Dr. G. W. C. Milner, F.R.I.C., A.Inst.P.

The subject of the meeting was "Nuclear Magnetic Resonance" and the following papers were presented and discussed: General Introduction, by R. E. Richards, M.A., D.Phil., F.R.S.; "The Application of Broad-line Nuclear Magnetic Resonance to Inorganic Analysis," by D. J. Ferrett, M.A., D. Phil.; "Applications of Nuclear Magnetic Resonance in Organic Chemistry," by L. M. Jackman, Ph.D. (see summaries below).

The meeting was preceded at 1.45 p.m. by a visit to the Analytical Chemistry Branch of the Atomic Energy Research Establishment, Harwell.

GENERAL INTRODUCTION

DR. R. E. RICHARDS briefly described the origin of nuclear resonance spectra. In solids the lines were often broad, but in liquids they were usually very narrow. The intensity of the nuclear resonance spectrum depended only on the number of nuclei causing it, and on no other property; it was independent of state and type of chemical combination. The author discussed the possibility of the quantitative analysis of mixtures of solids, and of solids and liquids, and described the inherent sensitivity of the method.

The spectra of liquids were often complex, because of the occurrence of chemical shifts and spin - spin coupling between nucleui. Dr. Richards considered the origin of these effects as well as the significance they had for qualitative and quantitative analysis of organic and inorganic substances.

THE APPLICATION OF BROAD-LINE NUCLEAR MAGNETIC RESONANCE TO INORGANIC ANALYSIS

DR. D. J. FERRETT said that, in the main, the application of nuclear magnetic resonance techniques to chemical analysis had been confined to the study of the spectra obtainable from organic molecules by high-resolution techniques, using magnets with highly homogeneous fields. Magnets with less homogeneous fields produced simpler spectra, and the use of these spectra for quantitative analysis had been investigated. He described the equipment that had been constructed and spoke on a number of applications to inorganic problems of analytical interest in liquid and solid systems.

APPLICATIONS OF NUCLEAR MAGNETIC RESONANCE IN ORGANIC CHEMISTRY

DR. L. M. JACKMAN said that the proton magnetic resonance spectra of organic compounds provided information that could be of considerable utility in both qualitative and quantitative analysis.

Qualitative applications—Dr. Jackman outlined the theory of proton chemical shifts and spin - spin coupling. He showed that chemical shift data could frequently be used to establish the presence or absence of functional groups, which hitherto had only been reliably characterised by chemical methods.

The way in which the spin - spin coupling constants were used to establish structural features in organic molecules was described, and an example of the combined use of chemical shift and spin - spin coupling data was given.

Quantitative applications—The intensity of a nuclear magnetic resonance absorption band was proportional to the number of nuclei that produced it. The determination of accurate intensities required considerable care, and the author outlined the principal experimental factors.

He drew attention to applications in which the nuclear magnetic resonance method might replace existing methods, *e.g.*, for methoxyl determination and C—Me determination.

Dr. Jackman also mentioned the application of nuclear magnetic resonance to specific analytical problems, such as the determination of the composition of mixtures of stereoisomers and of labile mixtures (*e.g.*, keto - enol equilibria).

A Procedure for Determining the Molar Extinction Coefficients of Metal Dithizonates*

By H. IRVING and R. S. RAMAKRISHNA
(*The Inorganic Chemistry Laboratory, South Parks Road, Oxford*)

Methods for evaluating the molecular extinction coefficients of metal complexes with dithizone are reviewed and a method of general applicability is proposed. The procedure is illustrated by the determination of the molecular extinction coefficients of silver and mercury dithizonates.

In recent years several derivatives of diphenylthiocarbazone, Ar·N:N·C(SH):N·NH·Ar', have been prepared and their visible absorption spectra and molecular extinction coefficients have been recorded.[1,2,3,4,5]

However, there has been little detailed study of the effects of substituents in the aryl residues (Ar and Ar') of substituted dithizones on the absorption spectra of the reagents or their metal complexes. Little is known about the effect of such substituents on the stability of metal - dithizone complexes, on their solubility in water and organic solvents or on the whole complex picture of their partition equilibria. Discussion of the relative sensitivities of substituted dithizones for determining traces of metals must rest heavily on the relevant values for molar extinction coefficients, and a general method for determining these is of obvious importance.

Reliable methods of obtaining the molecular extinction coefficients of dithizone and their derivatives include a direct method described by Weber and Vouk,[6] indirect methods described by Cooper and Sullivan[7] and a mercury titration procedure; these methods have been used in this laboratory for some time. However, the procedures hitherto described for determining the extinction coefficients of the metal - dithizone complexes are not suitable for all metals or even for all the various substituted dithizones. A more serious criticism is that they fail to take into account the effect of light-absorbing impurities in the reagent on the recorded values of the extinction coefficients.

Cooper and Sullivan's mono-colour method[7] for mercury and silver involves preparation of a solution of the metal dithizonate in a suitable organic solvent that contains excess of reagent, removal of the excess of reagent, measurement of the optical density of the supposedly pure dithizonate and computation of the extinction coefficient on the assumption that the metal has reacted stoicheiometrically with dithizone. The assumption that the concentration of the metal dithizonate is equal to that of the metal originally used will not hold, however, if the wash solution used to remove excess of reagent decomposes any of the metal complex; this is a weak feature of Cooper and Sullivan's procedure. Takei, Shibuya and Kato[8] applied this method to determine the extinction coefficients of the complexes of cadmium, mercury, zinc and lead with *pp'*-dimethyldithizone and, for lead, commented on the difficulty of finding a suitable wash solution to remove the excess of reagent. For metal complexes with substituted dithizones, therefore, the difficulty of finding wash solutions that will separate the excess of dithizone from the metal dithizonates without decomposing them cannot be overlooked if the method is to be of general applicability. The percentage of metal extracted is often very sensitive to the excess of reagent present in the organic phase[9] and only in exceptional instances can the technique of "stripping" the excess of reagent be justified theoretically or practically.

The mixed-colour method used by Cooper and Sullivan[7] for lead and zinc involves preparation of a solution of the metal dithizonate in a suitable organic solvent by equilibrating a solution of dithizone with an excess of metal. The optical density of the metal complex is then determined, and, after the organic portion of the complex has been removed, the amount of metal originally bound to the dithizone is determined by an independent absorptiometric dithizone procedure. The major disadvantage of the method lies in the choice of a suitable reversion reagent that will not interfere with the subsequent determination of the metal in the aqueous phase. There is the further possibility of multiplying relative errors, as the values of optical density and metal content used in evaluating the extinction coefficient of the metal complex will have been determined by two independent dithizone methods.

* Presented at the meeting of the Society on Wednesday, October 5th, 1960.

A direct method of determining the extinction coefficient of lead dithizonate has recently been described by Weber and Vouk.[6] Here, a solution of pure lead dithizonate containing no excess of dithizone is prepared, its optical density is measured and the lead content is then determined polarographically. No independent check on the purity of the lead dithizonate prepared was suggested, and the extension of the method to dithizone complexes of other metals would certainly not prove convenient.

The determination of molecular extinction coefficients from the optical densities of solutions containing known weights of previously prepared solid samples of the metal dithizonates is scarcely feasible, owing to the difficulty of preparing complexes of known composition. This is indicated by the difficulty of obtaining satisfactory analytical results for samples of dithizonates precipitated from aqueous solutions.[10] Because of their low solubilities in almost all solvents it is seldom possible to recrystallise a metal dithizonate; mercury dithizonate can be recrystallised from hot pyridine, but the crystals retain solvent tenaciously.[11] Further, many metal dithizonates are unstable to light and deteriorate on keeping.

The mercury titration procedure originally devised to determine the extinction coefficients of dithizone and its complex with mercury consists in equilibrating equal volumes of a solution of purified dithizone with aqueous solutions containing known amounts of mercury insufficient to react with all the dithizone present.

Let C_r and C_c be the concentrations of the excess reagent and metal complex, MR_n, respectively, and ϵ_r and ϵ_c their respective molecular extinction coefficients. Let the superscripts c and r designate absorption at the wavelength of maximum absorption of the complex and reagent, respectively. Then the optical density of the mixed-colour organic phase will be given by—

$$A^c = C_c\epsilon_c^c + C_r\epsilon_r^c + \Sigma\, C_i\epsilon_i^c \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$A^r = C_c\epsilon_c^r + C_r\epsilon_r^r + \Sigma\, C_i\epsilon_i^r \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where C_i represents the concentration of light-absorbing impurities whose partition coefficients favour the organic phase, and ϵ_i their molar extinction coefficients. If C_r^0 is the initial concentration of the reagent, that of the excess reagent in solution will always be $C_r^0 - nC_c$, since each molecule of metal complex MR_n corresponds to n molecules of reagent.

Equations (1) and (2) then become—

$$A^c = C_c(\epsilon_c^c - n\epsilon_r^c) + C_r^0\epsilon_r^c + \Sigma\, C_i\epsilon_i^c \quad \ldots\ldots\ldots\ldots \quad (3)$$

$$A^r = C_c(\epsilon_c^r - n\epsilon_r^r) + C_r^0\epsilon_r^r + \Sigma\, C_i\epsilon_i^r \quad \ldots\ldots\ldots\ldots \quad (4)$$

A plot of A^r against C_c will be a straight line of slope $(\epsilon_c^r - n\epsilon_r^r)$, and a graph of A^c against C_c will be a straight line of slope $(\epsilon_c^c - n\epsilon_r^c)$. When just enough metal has been added to form a complex with all reagent, *i.e.*, when $C_c = C_r^0/n$, equation (4) becomes—

$$A^r = \frac{C_r^0(\epsilon_c^r - n\epsilon_r^r)}{n} + C_r^0\epsilon_r^r + \Sigma C_i\epsilon_i^r$$

$$= \frac{C_r^0\epsilon_c^r}{n} + \Sigma\, C_i\epsilon_i^r \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

The intercept when $A^r = 0$ gives the amount of metal equivalent to the reagent in the original solution. If, however, $\epsilon_c^r \neq 0$ and, or if, the impurities absorb significantly at the wavelength of maximum absorption of the reagent, the plot of A^r against C_c will conclude with a section parallel to the abscissa and the value of C_c at the intersection of the two lines will give C_r^0/n. The extinction coefficient of the reagent is then calculated from the value of C_r^0 found in this way and from A^r for the original solution (see Fig. 1).

When $C_c = C_r^0/n$, equation (3) becomes—

$$A^c = \frac{C_r^0(\epsilon_c^c - n\epsilon_r^c)}{n} + C_r^0\epsilon_r^c + \Sigma C_i\epsilon_i^c$$

$$= \frac{C_r^0\epsilon_c^c}{n} + \Sigma C_i\epsilon_i^c \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

The extinction coefficient of the metal complex is calculated from the value of A^c when $C_c = C_r^0/n$.

The general utility of the above method for determining the extinction coefficients of other metal complexes with dithizone and its derivatives is limited by the significant absorption shown by many such metal dithizonates at the wavelength of maximum absorption of the

reagent. The method also assumes that the concentrations of the complexes formed are exactly equivalent to the concentrations of the metal solutions taken; further, it provides no check on spurious absorption by impurities at the wavelengths chosen.

A simple procedure is proposed in this paper; it can be applied to the evaluation of the extinction coefficients of metal complexes with dithizone and its derivatives. This would make possible comparison of values for a particular reagent with change of the metal used, the absolute values being evaluated if the extinction coefficient of the reagent is known and the relative values otherwise. The theory of this procedure is described below.

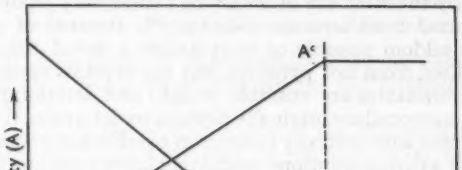

Fig. 1. Form of graphs used for calculating value of extinction coefficient

If A^c is the optical density at the wavelength of maximum absorption of a metal complex obtained by equilibrating a solution of the reagent in a water-immiscible solvent with an excess of metal ions, then—

$$A^c = C_c\epsilon_c^c + \Sigma C_i\epsilon_i^c \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

If the organic phase is then shaken with a reagent that will decompose only the metal complex, MR_n, permitting the return of metal ions to the aqueous phase while liberating an equivalent amount of reagent in the organic phase, the optical density at the wavelength of maximum absorption of the reagent will be given by—

$$A_R^r = nC_c\epsilon_r^r + \Sigma C_i\epsilon_i^r \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

where ϵ_c^c, C_c, ϵ_i^c, C_i, ϵ_r^r and ϵ_i^r have the meanings already ascribed to them and A_R^r is the optical density of the reverted organic phase at the wavelength of maximum absorption of the reagent.[12]

Since solutions of the pure metal and metal-free aqueous solutions of the reversion reagents are used, any coloured impurities present in the systems can be assumed to be derived only from the reagent and are hence present in amounts proportional to the concentration of the reagent used for forming the metal complex; this, in turn, will be proportional to the concentration of the reverted reagent, *viz.*, to nC_c.

In this instance, then—

$$\Sigma C_i\epsilon_i^c = nC_c\Sigma k_i\epsilon_i^c$$
$$\text{and } \Sigma C_i\epsilon_i^r = nC_c\Sigma k_i\epsilon_i^r \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (9)$$

where k_i represents the constant of proportionality.

All aqueous solutions used are pre-saturated with the organic solvent so as to minimise any possible changes in volume during equilibration and reversion.

From equations (7), (8) and (9)—

$$A^c = C_c\epsilon_c^c + nC_c\Sigma k_i\epsilon_i^c \quad .. \quad .. \quad .. \quad .. \quad .. \quad (10)$$
$$A_R^r = nC_c\epsilon_r^r + nC_c\Sigma k_i\epsilon_i^r \quad .. \quad .. \quad .. \quad .. \quad .. \quad (11)$$

and hence

$$A^e = A^r_E \left(\frac{\epsilon^e_e + n\Sigma k_i \epsilon^e_i}{n\epsilon^r_r + n\Sigma k_i \epsilon^r_i} \right) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (12)$$

Hence plots of A^e against A^r_E obtained by using different initial concentrations of the reagent should be linear and should pass through the origin. In the absence of any impurities the gradient would be given by $\epsilon^e_e/n\epsilon^r_r$. The main impurity in the dithizone is its oxidation product, the thiocarbodiazone, the absorption of which is strongest near or below the wavelength of maximum absorption of the metal complex and is negligible at the wavelength of maximum absorption of the dithizone. Hence the quotient

$$(\epsilon^e_e + n\Sigma k_i \epsilon^e_i)/(n\epsilon^r_r + n\Sigma k_i \epsilon^r_i)$$

will always be greater than $\epsilon^e_e/n\epsilon^r_r$.

All plots of A^e against A^r_E should yield straight lines passing through the origin, and their gradients should increase with the decrease in purity of the reagent used.

The proposed method was tested with mercury and silver by using dithizone solutions of different degrees of purity.

Method

Reagents—

All reagents were of recognised analytical grade.

Metal-free water—Pass laboratory distilled water through a mixed-bed ion-exchange resin.

Carbon tetrachloride—Redistil in an all-Pyrex-glass apparatus.

Ammonium hydroxide and hydrochloric acid solutions, dilute (1 + 100)—Prepare concentrated solutions isopiestically by Irving and Cox's procedure,[13] and suitably dilute with de-ionised water.

Dithizone solutions—Prepare a stock solution by dissolving about 100 mg of purified dithizone in 1 litre of redistilled carbon tetrachloride. Purify by Cowling and Miller's procedure,[14] and dilute the solution as required. Solutions of different and lower purity were obtained by allowing the purified diluted solutions to stand for periods of time exposed to light and air. The decrease in peak ratio $\epsilon_{620}/\epsilon_{450}$ indicated the gradual formation of oxidation products, and the purity of the reagent as used was calculated from the measured optical densities at 620 and 450 mμ and the known extinction coefficients for pure dithizone.[7]

Standard mercury solution—Dissolve mercuric chloride in 0·25 *N* sulphuric acid to give a concentration of $2{\cdot}1884 \times 10^{-3}$ mole per litre. Dilute this stock solution 1 to 100 with de-ionised water pre-saturated with carbon tetrachloride.

Reversion reagent for mercury—Dissolve 10 g of potassium hydrogen phthalate and 30 g of potassium iodide in 500 ml of de-ionised water, and add a few drops of sodium thiosulphate solution. Purify by shaking with portions of a solution of dithizone in redistilled carbon tetrachloride (approximately 50 mg per litre) until two successive extracts show the unchanged green colour of the reagent. Shake the reversion reagent with two small portions of redistilled carbon tetrachloride to extract any dissolved dithizone. Saturate this resulting solution with carbon tetrachloride.

Standard silver solutions—Dissolve silver nitrate in 0·25 *N* sulphuric acid to a concentration of $3{\cdot}0819 \times 10^{-3}$ mole per litre. Before each determination, dilute this stock solution 1 to 50 with de-ionised water pre-saturated with carbon tetrachloride.

Reversion reagent for silver—Decolorise a 6 per cent. w/v solution of potassium iodide in 0·25 *N* sulphuric acid by adding a few drops of sodium thiosulphate solution. Purify as described for the reversion reagent for mercury, and saturate with carbon tetrachloride.

Apparatus—

All apparatus of Pyrex glass must be specially cleaned by soaking in chromic - sulphuric acid mixture and then rinsing with warm tap-water and finally with distilled water. The washing procedure is then repeated with warm diluted nitric acid (1 + 1), distilled water and de-ionised water, in that order. The efficiency of the cleaning process should always be tested with a drop of dilute dithizone solution.

Stoppered Pyrex-glass tubes were used for equilibration and pear-shaped Pyrex-glass 100-ml separating funnels for reversion.

Optical densities were measured in 1-cm fused-silica cells with a Unicam SP500 spectrophotometer.

PROCEDURE—

Ten-millilitre portions of dithizone solutions of different concentrations prepared from x ml of purified dithizone solution and $(10 - x)$ ml of pure carbon tetrachloride were shaken mechanically for 10 minutes with 10 ml of the metal solution in stoppered tubes protected from light by being wrapped in black paper. The organic phase was spun in a centrifuge to free it from water droplets, and the optical density was measured at the wavelengths of maximum absorption of the metal complex and of the dithizone. A 5-ml portion of this organic extract was then placed, by pipette, in a 100-ml separating funnel and shaken with 5 ml of the reversion reagent. The organic phase was then withdrawn through a pledget of metal-free cotton-wool, and its optical density was measured at 620 and 450 mμ. This procedure was repeated with solutions of dithizone of different purity. The mole ratios of metal to reagent were always sufficiently large to bring about quantitative complexation of all the reagent. The fact that light-absorbing adventitious impurities were not introduced during equilibration and reversion was shown by the constancy (within limits of experimental error) of the values of the peak ratios, $\epsilon_{620}/\epsilon_{450}$, of the reverted dithizone.

RESULTS

Independent measurements of A^c and A^r_g for four solutions of dithizone of different degrees of purity were made for the mercury dithizonate and with three solutions of dithizone for the silver dithizonate.

TABLE I

OPTICAL-DENSITY VALUES FOR MERCURY DITHIZONATE SYSTEM

Volume of purified dithizone solution used, ml	Optical density at 490 mμ	Optical density of reverted dithizone at 620 mμ	Ratio of ϵ_{620} to ϵ_{450} for reverted dithizone
(*a*) *Ratio of* ϵ_{620} *to* ϵ_{450} = 1·674; *purity of dithizone* = 98·30 *per cent.*—			
10	1·186	1·136	1·671
9	1·068	1·000	1·669
8	0·920	0·890	1·670
7	0·809	0·758	1·668
6	0·553	0·532	1·672
5	0·478	0·445	1·673
4	0·440	0·415	1·670
(*b*) *Ratio of* ϵ_{620} *to* ϵ_{450} = 1·620; *purity of dithizone* = 95·13 *per cent.*—			
8	0·984	0·892	1·619
7·5	0·924	0·850	1·616
6	0·741	0·680	1·616
4	0·495	0·451	1·618
3·5	0·441	0·397	1·614
2	0·255	0·217	1·607
(*c*) *Ratio of* ϵ_{620} *to* ϵ_{450} = 1·458; *purity of dithizone* = 85·62 *per cent.*—			
10	1·131	1·012	1·454
9	1·016	0·905	1·452
8	0·893	0·835	1·450
7	0·796	0·727	1·448
6	0·680	0·616	1·453
5	0·583	0·515	1·451
4	0·470	0·401	1·448
3	0·358	0·309	1·443
(*d*) *Ratio of* ϵ_{620} *to* ϵ_{450} = 1·299; *purity of dithizone* = 76·28 *per cent.*—			
8	1·049	0·920	1·283
7	0·908	0·800	1·278
6	0·783	0·660	1·280
5	0·646	0·562	1·272
4	0·524	0·452	1·269
3	0·400	0·330	1·267
2	0·268	0·214	1·265

TABLE II

EXTINCTION COEFFICIENTS FOR MERCURY DITHIZONATE

Purity of dithizone, %	Intercept	Gradient	Value of ϵ_{490} for mercury dithizonate*	Value of "t" for intercept	Critical value of "t" for 95 per cent. confidence limits
98·30	0·008	1·0435	72,210	0·503	2·571
95·13	0·017	1·0718	74,168	1·027	2·776
85·62	0·018	1·0858	75,137	1·253	2·447
76·28	0·033	1·1047	76,445	1·369	2·571

* Assuming no impurities in dithizone.

TABLE III

OPTICAL-DENSITY VALUES FOR SILVER DITHIZONATE SYSTEM

Volume of purified dithizone solution used, ml	Optical density at 462 mμ	Optical density of reverted dithizone at 620 mμ	Ratio of ϵ_{620} to ϵ_{450} for reverted dithizone
(*a*) *Ratio of* ϵ_{620} *to* ϵ_{450} = 1·664; *purity of dithizone* = 97·71 *per cent.*—			
10	1·165	1·360	1·652
9	1·085	1·233	1·658
8	0·986	1·099	1·660
7	0·864	0·971	1·650
6	0·752	0·836	1·661
5	0·630	0·679	1·649
4	0·480	0·538	1·654
3	0·381	0·412	1·653
(*b*) *Ratio of* ϵ_{620} *to* ϵ_{450} = 1·617; *purity of dithizone* = 94·95 *per cent.*—			
10	1·290	1·482	1·610
9	1·221	1·354	1·612
8	1·091	1·213	1·613
7	0·946	1·029	1·601
6	0·824	0·885	1·598
5	0·668	0·730	1·607
4	0·543	0·603	1·587
3	0·370	0·405	1·602
(*d*) *Ratio of* ϵ_{620} *to* ϵ_{450} = 1·448; *purity of dithizone* = 85·03 *per cent.*—			
10	0·890	0·965	1·440
9	0·850	0·900	1·439
8	0·742	0·782	1·434
7	0·642	0·666	1·442
6	0·546	0·563	1·430
5	0·407	0·414	1·432

TABLE IV

EXTINCTION COEFFICIENTS FOR SILVER DITHIZONATE

Purity of dithizone, %	Intercept	Gradient	Value of ϵ_{462} for silver dithizonate*	Value of "t" for intercept	Critical value of "t" for 95 per cent. confidence limits
97·71	0·043	0·8416	29,118	2·225	2·447
94·95	0·038	0·8656	29,951	1·279	2·447
85·03	0·049	0·8807	30,472	1·129	2·776

* Assuming no impurities in dithizone.

Optical-density values for the mercury and silver dithizonate systems are shown in Tables I and III, respectively, and plotted in Fig. 2. Tables II and IV show the values of the extinction coefficients of the complexes with mercury and silver, respectively. The "t" values for the intercepts calculated from measurements with dithizone solutions of various

purities are quoted with those for the 95 per cent. confidence limits. The values for the extinction coefficients of the mercury and silver dithizonates and the purity of the dithizone solutions are calculated from the values quoted by Cooper and Sullivan[7] for pure dithizone.

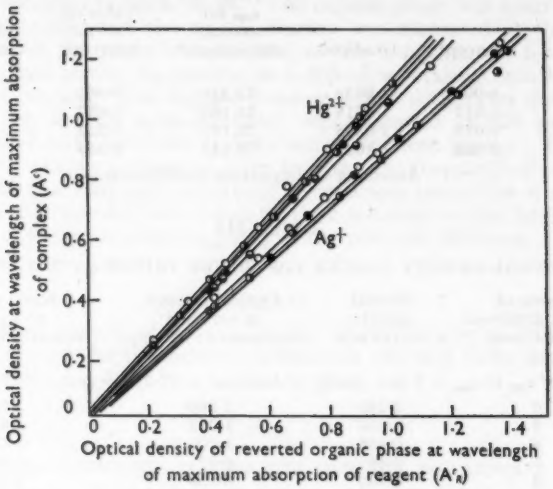

Fig. 2. Graphs of optical-density values shown in Tables I and III

Discussion of results

The plots of A^c against A_R^{620} (see Fig. 2) were linear over the optical-density range 0·25 to 1·25. Very small positive intercepts were sometimes noted, but these were shown to be statistically insignificant (see Tables II and IV) by regression analysis,[15,16,17] which indicated that the intercepts differed from zero by no more than could be accounted for by analytical errors. The gradients were found to increase with decreasing purity of the reagent used, as expected from the form of equation (12). However, the molecular extinction coefficients obtained experimentally when a sample of dithizone having a purity of only about 85 per cent. was used is less than 5 per cent. greater than the value obtained with the purest available reagent. Since solutions of dithizone having a purity above 95 per cent. can easily be prepared by Cowling and Miller's method,[14] satisfactory values for the extinction coefficients of its metal complexes can be obtained even when the purity of the reagent is not 100 per cent.

The relative ease with which reversion reagents for all metal - dithizone complexes can be obtained, based on the extensive qualitative data available on the stability of various metal complexes in the presence of hydrogen ions or anions, provides the basis for developing the method into one of general applicability. It will be realised that the proposed method should be equally applicable to other metal - reagent systems.

One of us (R.S.R.) expresses his gratitude to the Council of the University of Ceylon for providing facilities for study leave.

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The Spectrophotometric Determination of Microgram Amounts of Calcium*

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A procedure has been developed for determining calcium with glyoxal bis-(2-hydroxyanil). In alkaline solution, calcium reacts with the reagent to form a red complex, which is stable in an aqueous alcoholic medium and has an absorption maximum at 520 mμ. The colour obeys Beer's law in the range 0 to 4 p.p.m. of calcium, and no interference is encountered from up to 50 p.p.m. of magnesium.

In waters of low mineral content, such as rain water, calcium is usually determined by flame photometry. This is a relatively insensitive method, and, in practice, errors of the order of 0·5 p.p.m. must be accepted. A search of the literature reveals few available colorimetric methods; Sandell[1] quotes five in detail. Two of these are indirect, *e.g.*, one involves determination of excess of oxalate by a ceric salt,[2] a multiple-stage micro-technique being used, and the other involves determination of phosphate[3] by the molybdenum-blue method after removal of magnesium. Chloranilate[4] is only suitable for milligram amounts of calcium. Murexide[5] is sensitive, and the presence of up to 10 p.p.m. of magnesium is permissible when this reagent is used, but it is extremely unstable. *o*-Cresolphthalein complexone[5] has been used for determining calcium after removal of magnesium by paper chromatography,[6] but the calibration graph is non-linear.

Goldstein and Stark-Mayer[7] have described a qualitative test for microgram amounts of calcium, based on the reaction between an alkaline solution of calcium and glyoxal bis-(2-hydroxyanil).

Experimental

Goldstein and Stark-Mayer's test has been investigated. When the alkaline solution of calcium and glyoxal bis-(2-hydroxyanil) is extracted with chloroform, a red colour in the chloroform layer indicates the presence of calcium. Efforts to obtain quantitative results by increasing the volumes of sample and reagents and measuring the optical densities of the chloroform extracts of the calcium - glyoxal bis-(2-hydroxyanil) complex were unsuccessful. The complex is insoluble in water, but dissolves in various organic solvents. It was found that, by omitting the extraction stage and carrying out the reaction in an aqueous alcoholic medium, red colours were formed. The intensity of the colour was proportional to the amount of calcium present, but the stability was poor and fading occurred within several minutes. The colour was eventually stabilised by substituting an alkaline buffer solution for the cyanide and carbonate reagents used by Goldstein and Stark-Mayer.[7]

Fig. 1 shows the absorption spectra of the reagent and of its complex with calcium, the optical-density measurements being made with a Unicam SP600 spectrophotometer. To determine the optimum test conditions and ascertain the stability of the colour, time - development curves were plotted at 520 mμ. These curves are shown in Fig. 2, from which it can be seen that the colour is fully developed after 25 minutes and is stable for at least 1 hour. The colour obeys Beer's law in the range 0 to 40 μg of calcium.

* Presented at the meeting of the Society on Wednesday, October 5th, 1960.

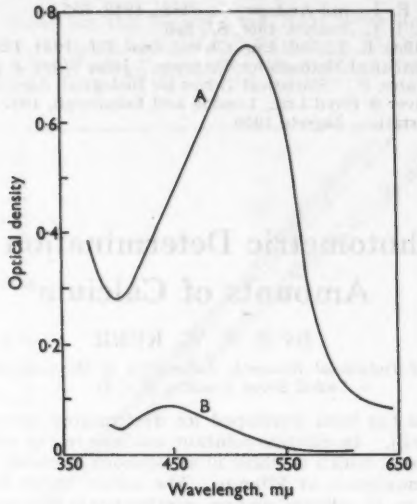

Fig. 1. Absorption spectra: curve A, calcium complex of glyoxal bis-(2-hydroxyanil); curve B, reagent. Readings were made in 1-cm cells against distilled water

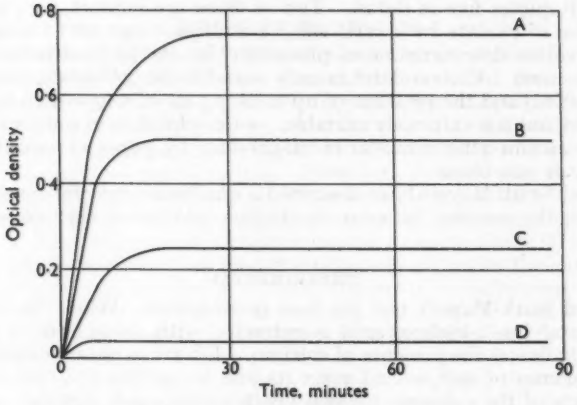

Fig. 2. Development of colour for different amounts of calcium: curve A, 30 μg; curve B, 20 μg; curve C, 10 μg; curve D, 0 μg. The reference cell contained distilled water

EFFECT OF pH—

No colour development took place in solutions having pH values lower than 11·8, and change in pH over the range 12·2 to 12·6 had little effect on colour formation. Above this range of values, the complex was unstable, and a brown background colour developed.

EFFECT OF TEMPERATURE—

Temperatures ranging from 10° to 30° C had no effect on colour development. At 5° C, however, the rate of development was slow, and the absorption maximum was reached only after 40 minutes.

EFFECT OF SOLVENT—

The effects of various organic solvents on the stability of the colour were studied. The presence of acetone and *iso*propyl alcohol led to gradual decomposition of the complex. Ethanol, methanol and a mixture of ethanol and *n*-butyl alcohol all gave good colour stability, the last-named being slightly superior to the other two.

INFLUENCE OF FOREIGN IONS—

Magnesium and aluminium in concentrations up to 50 p.p.m. do not interfere. Barium and strontium cause interference at a level of 4 p.p.m. Ferrous iron in concentrations up to 50 p.p.m. does not interfere, provided that sufficient potassium cyanide is present to prevent precipitation of ferrous hydroxide. Sulphate, chloride and nitrate in concentrations up to 500 p.p.m. have no effect.

METHOD

Water used for dilution purposes and in the preparation of reagent solutions should be distilled and then de-ionised by passage through a column of ion-exchange resin.

REAGENTS—

Buffer solution, pH 12·6—Dissolve 10 g each of sodium hydroxide and sodium tetraborate in water, and dilute to 1 litre.

Glyoxal bis-(2-hydroxyanil) reagent solution—Dissolve 4·4 g of freshly sublimed *o*-aminophenol in 1 litre of water at 80° C, add 3·5 ml of glyoxal solution (30 per cent. w/w), and maintain at 80° C for 30 minutes. Cool, and set aside for 12 hours, preferably in a refrigerator. Separate the precipitate by filtration, wash with water, and recrystallise from methanol. The yield is approximately 2 g of glyoxal bis-(2-hydroxyanil). Prepare a 0·5 per cent. w/v solution of the reagent in methanol.

Solvent mixture—Mix equal volumes of ethanol and *n*-butyl alcohol.

Standard calcium solution—Dissolve 1·001 g of calcium carbonate in 25 ml of *N* hydrochloric acid, and dilute to 1 litre. Dilute 5 ml of this stock solution to 500 ml.

1 ml ≡ 4 μg of calcium.

PROCEDURE—

Transfer a 10-ml aliquot of sample containing not more than 40 μg of calcium to a stoppered cylinder. Add 1·0 ml of buffer solution, 0·5 ml of glyoxal bis-(2-hydroxyanil) reagent solution and 10 ml of solvent mixture (in that order), and mix after each addition. Set aside for 30 minutes, and then measure the optical density in 1-cm cells with a spectrophotometer at 520 mμ or with an absorptiometer and Ilford No. 604 (green) filters; use distilled water as reference solution.

TABLE I

CALCIUM CONTENTS OF SAMPLES OF RAIN WATER

Date	Site of collection	Calcium content found by— titration against EDTA,* p.p.m.	proposed method, p.p.m.
December, 1959	Edinburgh	1·1	1·0
	Leeds	1·3	1·2
	Harpenden	0·8	0·7
January, 1960	Aberdeen	1·0	1·01
	Edinburgh	1·1	1·06
	Leeds	0·6	0·54
	Harpenden	1·2	1·20
	Newton Abbot	1·2	1·17
	Lerwick	6·8	6·96
	Stornoway	2·4	2·25
	Eskdalemuir	0·2	0·11
February, 1960	Aldergrove	1·2	1·1
	Eskdalemuir	0·3	0·25
	Lerwick	3·9	3·9
	Stornoway	1·1	1·2
	Newton Abbot	1·5	1·6

* The samples for titration were obtained by evaporating 500 ml of rain water to 50 ml.

Results

The proposed method was applied to solutions prepared by diluting aliquots of standard calcium solution with distilled water; the results were—

Calcium present, p.p.m.	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
Calcium found, p.p.m.	0·13	0·41	0·28	0·40	0·50	0·62	0·70	0·83	0·95

The calcium content of a sample of sea water (440 p.p.m.) was determined by titration against ethylenediaminetetra-acetic acid (EDTA), with murexide as indicator. Aliquots of this sea water were diluted with distilled water, and the calcium contents of the dilutions were determined by the proposed method; the results were—

Calcium present (calculated), p.p.m.	1·32	1·41	1·50	1·58	1·67	1·76	2·64	2·82	3·00	3·17	3·35	3·52
Calcium found, p.p.m.	1·36	1·40	1·49	1·53	1·70	1·85	2·65	2·76	2·88	3·16	3·32	3·52

Table I shows a comparison between the results found for calcium in samples of rain water by the proposed method and by titration against EDTA.

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The Determination of Boron in Silicon by Isotope Dilution*

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A method is described for determining traces of boron in silicon. The sample is dissolved in aqueous sodium hydroxide in the presence of ^{10}B tracer, and the boron is separated by a modification of Morrison and Rupp's method of electrolysis through a cation-exchange membrane.

The boron is finally determined by making isotope-dilution measurements with a mass spectrometer with a thermal-ionisation source.

The method has the advantage that quantitative separation of boron is not necessary. Amounts of boron down to 0·001 μg can be determined to within $\pm$30 per cent.

The presence of extremely small amounts of boron in silicon can greatly alter its electrical properties. Previous work on the determination of this impurity in silicon has been carried out by Luke,[1] Morrison and Rupp,[2] Luke and Flaschen[3] and Gill.[4] The last-mentioned worker determined boron down to the 0·003 p.p.m. level by proton activation of standard samples. This paper describes work on the application to this problem of isotope-dilution analysis with a mass spectrometer.

Isotope-dilution analysis

The principles of the method have been described in several reviews[5,6,7,8,9]; the technique has been used in the field of geochemistry and recently reviewed by Webster.[9] The method consists in taking a definite weight of sample into solution in the presence of a known amount of tracer. The tracer is a form of the element to be determined having an isotopic composition

* Presented at the meeting of the Society on Wednesday, November 2nd, 1960.

different from that of the natural element. Owing to the natural element present in the sample as impurity the isotopic composition of the tracer will be altered. After chemical separation, this alteration can be measured with a mass spectrometer and related to the amount of impurity. This is shown in Fig. 1 for boron, which has two isotopes, ^{10}B and ^{11}B.

The impurity content is calculated from the equation—

$$E = \frac{R^0 - R^1}{R^1 - R} \times \frac{R + 1}{R^0 + 1} \times \frac{A}{A^0} \times \frac{W}{M} \quad .. \quad .. \quad .. \quad (1)$$

in which E is the concentration of the element sought (in parts per million by weight); R, R^0 and R^1 are the isotopic ratios (^{10}B to ^{11}B) of the natural element, the enriched element added and the extracted mixture, respectively; A and A^0 are the atomic weights of the natural and enriched element, respectively; W is the weight of tracer added (in micrograms) and M is the weight of sample taken (in grams).

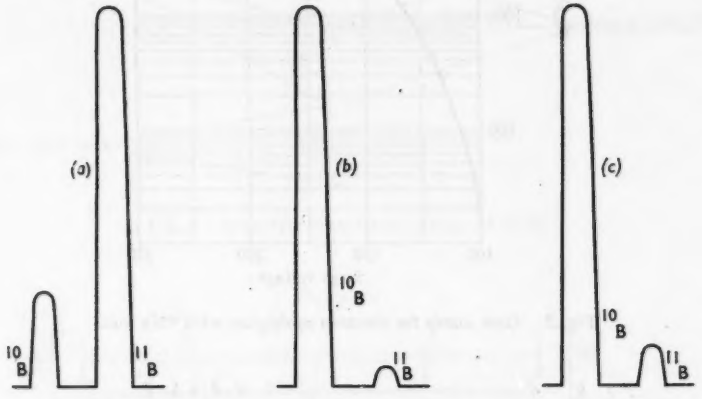

Fig. 1. Isotope ratios showing dilution of ^{10}B tracer: (*a*) natural element; (*b*) enriched tracer; (*c*) final mixture

For the measurement of isotope ratios with a mass spectrometer the residue obtained after separation must be in a suitable ionisable form. The ion $(Na_2BO_2)^+$ was used by Palmer[10] for measurements of isotope abundance, and the peaks corresponding to m/e values of 88 and 89 for this ion were used in this work. The separation was carried out by dissolving the silicon in sodium hydroxide solution with ^{10}B tracer present, electrolysing through a cation-exchange membrane to remove sodium ions and then evaporating the anolyte to dryness. The "crystalline" silicic acid obtained was leached with water to extract boron as sodium borate.

Mass spectrometer

A Metropolitan - Vickers MS2S instrument (see *Analyst*, 1954, **79**, facing p. 600) was used throughout this work. It has facilities for examining solid compounds by a thermal-ionisation technique. The sample in the form of a solution was loaded on to a tungsten-strip filament, the temperature of which varied with the current passing through it; hence the emission of positive ions from the filament could be controlled.

When the standard plate collector was used, the sensitivity was found to be insufficient for measuring the very small ion currents emitted by sub-microgram amounts of boron. The plate collector was therefore replaced by an end-on Allen Baxter electron multiplier[11] constructed from data supplied by G. H. Palmer, of the Atomic Energy Research Establishment, Harwell. (The instrument fitted with an electron multiplier is now available from Associated Electrical Industries Ltd.) The gain curve for a recently prepared electron multiplier is shown in Fig. 2. With this modification, the sensitivity for ion-current measurement was improved from 4×10^{-14} amp to 5×10^{-17} amp. The amplified output of the system was displayed on a chart recorder.

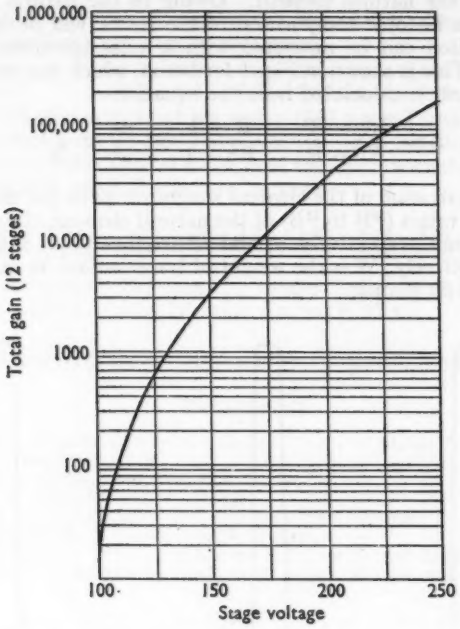

Fig. 2. Gain curve for electron multiplier with ^{23}Na ions

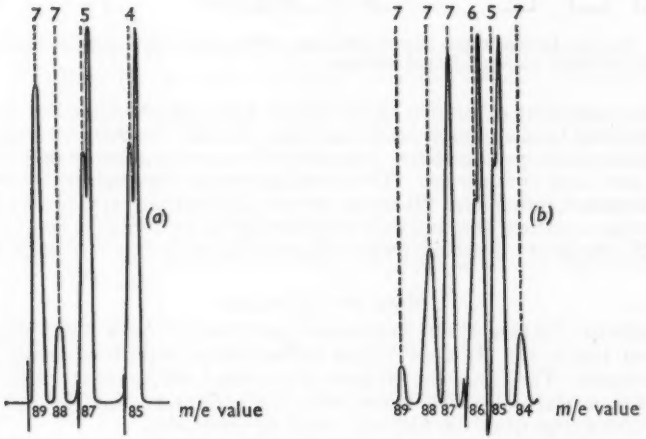

Fig. 3. Peaks from natural sodium tetraborate. Filament current: (*a*) 2·2 amps; (*b*) 4·5 amps

When boron, in the form of sodium tetraborate, is examined with a mass spectrometer fitted with an electron multiplier, a residual background of peaks is revealed due to the alkali metals, alkaline-earth metals and hydrocarbon cracking patterns. The hydrocarbon peaks can be removed by the inclusion of an extra cold trap containing liquid nitrogen, and, by suitable control of filament heating, the other interfering peaks can be reduced to negligible

proportions. Fig. 3 shows the peaks due to natural boron at m/e values of 88 and 89 from ions $(Na_2{}^{10}BO_2)^+$ and $(Na_2{}^{11}BO_2)^+$ and the interference from strontium when a higher filament current is used. Fig. 4 shows the various ions contributing to the spectrum over the m/e range 84 to 89 when the residual background is present.

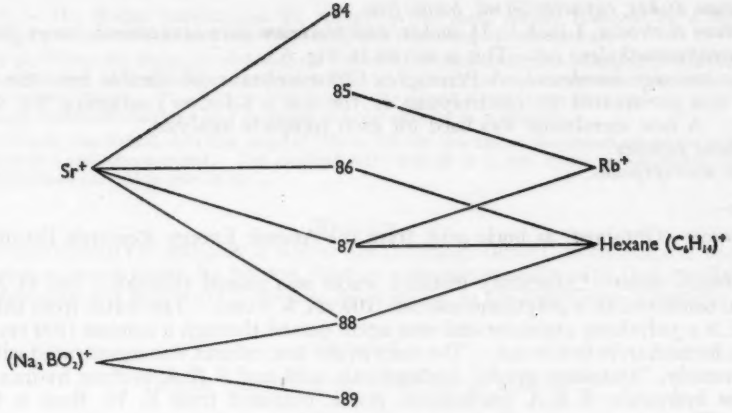

Fig. 4. Mass spectrum for m/e range 84 to 89

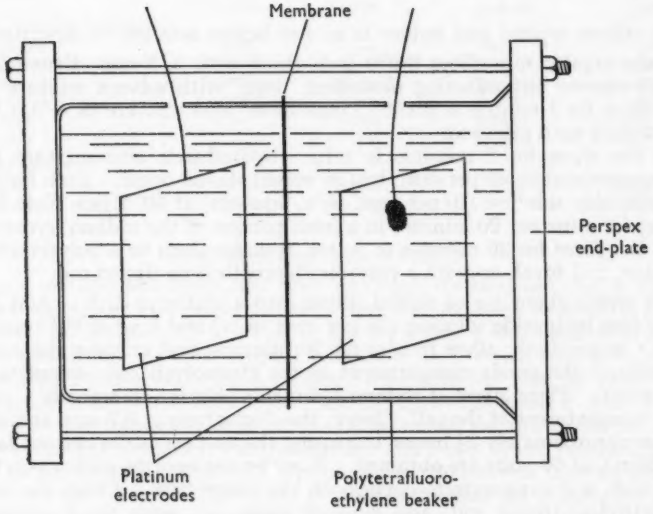

Fig. 5. Diagram of electrolysis cell

The rubidium peaks at m/e values of 85 and 87 do not affect the measurement of boron ratios and can, in fact, be used as "markers" when samples of boron having unknown isotopic compositions are being examined. Further, the presence of large amounts of alkali remaining in the residue after extraction inhibits the production of good peaks for boron, and it has been found imperative to pre-heat the loaded filament at a temperature lower than that required for ion emission until the excess of solids has volatilised.

Method

APPARATUS—

Platinum ware (obtainable from Johnson, Matthey & Co. Ltd.) was anodically cleaned before use by electrolysis with a platinum cathode in a 10 per cent. w/v solution of sodium hydroxide for 15 minutes at 1 amp.

Platinum dishes, capacity 50 ml, boron-free.

Platinum electrodes, 1 inch × 1½ inches, and platinum-wire connections, boron-free.

Polytetrafluoroethylene cell—This is shown in Fig. 5.

Cation-exchange membrane—A Permaplex C20 membrane (obtainable from the Permutit Co. Ltd.) was pre-treated by electrolysing in the cell a solution containing 6 g of sodium hydroxide. A new membrane was used for each complete analysis.

Polythene pipettes.

Quartz micropipettes.

REAGENTS—

^{10}B *Tracer*—Obtained, as boric acid, from the Atomic Energy Research Establishment, Harwell.

High-purity water—Laboratory distilled water was passed through a bed of Amberlite MB–1 resin contained in a polythene column (100 cm × 8 cm). The water from this column was stored in a polythene aspirator and was again passed through a column (100 cm × 4 cm) of the resin immediately before use. The resin in the last column was regenerated with special reagents, namely, "transistor-grade" hydrochloric acid and E.K.A. sodium hydroxide.

Sodium hydroxide—E.K.A. purissimum grade, obtained from F. W. Berk & Co. Ltd., was used. The boron content of this reagent was decreased before use by pre-electrolysis of an aqueous solution through the cleaned cation-exchange membrane. Sufficient reagent was prepared to carry out the analysis and the associated blank determination.

PROCEDURE—

Clean the silicon crystal and reduce it in size before solution as described below.

Cut the crystal into slices 0·020 inch thick with a Neven diamond-impregnated wheel, and remove any adhering mounting "wax" with solvent mixture (see Note 1). Etch the slices for 1 minute in nitric - hydrofluoric acid mixture (8 + 5, v/v), and store under methanol until required.

Etch the slices for 2 minutes in nitric - hydrofluoric acid mixture (8 + 5, v/v), when an approximately 40 per cent. loss in weight should occur. Etch for 20 minutes in sodium hydroxide solution (10 per cent. w/v, aqueous) at 50° C (see Note 2), and repeat the etching for a further 20 minutes in a fresh portion of the sodium hydroxide solution. Then boil the slices for 30 minutes in water, transfer them to a polytetrafluoroethylene 50-ml beaker, and break up with a polytetrafluoroethylene-tipped rod.

Accurately weigh about 1 g of etched silicon into a platinum dish. Add 20 ml of pre-electrolysed sodium hydroxide solution (10 per cent. w/v) and 1 μg of ^{10}B tracer. Heat for 30 minutes on a steam-bath, allow to cool for 20 minutes, and transfer the solution with a polythene pipette to the anode compartment of the electrolysis cell; complete the transfer with 10 ml of water. Place 30 ml of sodium hydroxide solution (nominally 1 per cent. w/v) in the cathode compartment of the cell. Begin the electrolysis at 0·7 amp and about 5 volts, and continue for approximately 2½ hours, increasing the voltage as the current falls until final conditions of 25 mA at 50 volts are obtained. Transfer the anolyte with a polythene pipette to a platinum dish, and evaporate to dryness on the steam-bath. Crush the residue with a polytetrafluoroethylene-tipped rod, add 2 ml of water, set aside for 5 minutes at room temperature, and transfer the solution to a second platinum dish. Evaporate to dryness on the steam-bath. Extract the final residue with the minimum amount of water (about 0·01 ml), and transfer with a quartz micropipette to the filament of the instrument. Insert the loaded filament into the source head, and replace the latter on the mass-spectrometer tube. Evacuate to about 5×10^{-7} mm of mercury, and pre-heat the filament for up to 1 hour by passing a current of 1·5 to 2 amps, depending on its thickness, through it.

Increase the filament current until good peaks for boron at m/e values of 88 and 89 are observed. Measure a series of not less than ten sets of peaks, and calculate the abundance ratio, R^1. Also determine the ratio R by using a solution of natural sodium tetraborate

and R^0 by using an alkaline solution of the tracer (see Note 3). Substitute the values found in equation (1) to ascertain the amount of boron present.

Determine the blank value for boron by carrying out the procedure described above, without the sample, immediately before analysing the silicon; use the same cell and membrane.

NOTES—

Note 1—The shellac cement used for mounting was most readily removed by a mixture containing equal volumes of acetone, ethyl methyl ketone and *cyclo*hexanone.

Note 2—When the slices are etched in 10 per cent. sodium hydroxide solution in the ordinary way, the hydrogen evolved causes the slices to rise to the surface, which prevents intimate contact with the etching liquid. This is overcome by immersing the slices in the dish under a perforated disc of polytetrafluoroethylene and carrying out the etching in a vacuum.

Note 3—It was found that the ratio of ^{10}B to ^{11}B for the tracer decreased when the solution was stored in a polythene vessel. The original ratio was 65 to 1, but when the results in Table I were determined the value was 54 to 1.

RESULTS

The boron contents of samples of silicon are shown in Table I, from which it can be seen that the boron content found by isotope dilution agrees nominally with that predicted from physical measurements and bears a suitable relationship to the resistivity, when this is given.

TABLE I

BORON CONTENTS OF SAMPLES OF SILICON

Description of sample	Nominal boron content, p.p.m.	Resistivity, ohms cm	Boron content found— from physical measurements, p.p.m.	Boron content found— by isotope dilution, p.p.m.
Single-crystal samples, p-type—				
Standard silicon 1	0·03	—	0·03	0·028
Standard silicon 2	0·01	—	0·01	0·019, 0·013
Standard silicon 3	0·003	40 to 27	0·003	0·002, 0·002
Standard silicon 4	0·003	24 to 18	0·003	0·005
Silicon SK665	—	160	0·0007	0·001, 0·001
Silicon SK959	—	250	0·0004	<0·001, <0·001
Polycrystalline sample—				
Silicon SK665	—	—	—	0·010, 0·018, 0·015

DISCUSSION OF THE METHOD

The proposed method was essentially that developed by Morrison and Rupp,[2] who used an emission spectrograph. The extraction technique was modified as described below.

A polytetrafluoroethylene cell (see Fig. 5) having a larger and vertical cation-exchange membrane was substituted for the original polythene cell, which had a small horizontal membrane; a similar Perspex cell with a vertical membrane was used by Logie.[12]

A higher current density was used to decrease the time of electrolysis from 5 to 2¼ hours; this also prevented the formation of a layer of silicic acid on the platinum anode. The electrolysis was taken further than it was by Morrison and Rupp, which resulted in less sodium ions remaining in the anolyte. The final weight of the residue was thereby decreased from about 10 mg to less than 1 mg. This made it possible to load the greater part of the final residue on to the relatively small area of the MS2S filament.

The presence of 1 μg of boron tracer, added initially, acted as a carrier for the sub-microgram amounts of natural boron present in the sample of silicon. The loss of boron during separation had less effect than in the original spectrographic method, in which only sub-microgram amounts of boron were handled. Also, separation of boron from silicon did not have to be quantitative, as the tracer was present throughout and any loss of natural boron would be expected to be accompanied by an equivalent loss of tracer. Although "isotope effects" can occur with light elements, the agreement between the nominal and determined boron contents of the standard silicons indicates that the chemical process did not discriminate between the two isotopes of boron.

During the early stages of this work, high blank values (about 0·2 μg of boron) were found, and this was traced to extraction from the platinum ware. When boron-free platinum was used, the blanks fell to a value of 0·02 μg, of which 0·005 μg was due to the water used in the analysis and to the platinum dish. This contribution was decreased to below the limit of detection (0·0007 μg) when the platinum ware was electrolytically cleaned. Further improvement was effected by pre-electrolysing the sodium hydroxide and the cation-exchange membrane. In the final method, the total blank was between 0·001 and 0·004 μg. A series of tests was carried out to establish the consistency of the blank, and it was found that, if two blank determinations were made on the same day, the values were nominally identical, the difference being less than 0·001 μg. However, such consistency was only attained if the determinations were made under rigidly controlled conditions and the manipulations were carried out in a form of dry box in a pressurised laboratory.

Solution of silicon in aqueous sodium hydroxide is only complete within a reasonable time if the sample is in the form of a fine powder. The use of an ostensibly boron-free die-steel pestle and mortar for crushing pure silicon led to high results, which could only be attributed to boron contamination from the pestle and mortar. Attempts were made to crush the sample without contaminating it by using pestles and mortars made of alumina and silver steel. Also, mortars lined with copper and aluminium were used, the sample being subsequently treated with suitable etching solutions to remove any mortar material adhering to the silicon. These methods generally introduced amounts of boron up to a maximum of 0·1 μg. The best solution to the problem was to crush very thin slices of silicon in a polytetrafluoroethylene beaker as described under "Procedure."

SENSITIVITY—

Examination of Fig. 1 and equation (1) shows that, for highest sensitivity, the ratio of ^{10}B to ^{11}B in the tracer, R^0, should be as high as possible, that the weight of tracer added, W, should be a minimum and the weight of sample, M, a maximum. The maximum ^{10}B content found in the tracer was 98·5 per cent., which corresponds to a ^{10}B to ^{11}B ratio of 65 to 1. The minimum weight of tracer found to be practical was 1 μg, and a weight of sample exceeding 1 g was inconvenient in practice. For a single sample, a typical set of ratios of ^{10}B to ^{11}B was 40·5 to 1, 40·4 to 1, 40·3 to 1, 40·0 to 1, 40·0 to 1, 39·9 to 1, 39·8 to 1, 39·7 to 1 and 39·5 to 1 (average, 40·0 to 1).

It can be seen that the over-all range is $\pm$1·25 per cent., but, when conditions are ideal, it is possible to measure the ratio to within $\pm$1 per cent. If the precision of measurement is 2 per cent. and if a change of 3 per cent. is considered to be significant, then the sensitivity is the amount of natural boron that would cause a 3 per cent. decrease in the ratio of ^{10}B to ^{11}B for the tracer. When values of 65 to 1 for R^0 and 1 μg for W are substituted in equation (1), the sensitivity is 0·0007 μg of boron.

However, in the analysis of silicon the blank value obtained governs the sensitivity, and the limit will be a significant difference of 3 per cent. in the ratio from that of the blank. Under the conditions applying for the results for boron in Table I, the sensitivity was found to be 0·001 μg.

The accuracy of the method alters with the level of boron from within $\pm$30 per cent. for 0·001 μg of boron to within $\pm$1 per cent. for 0·1 μg of boron.

We thank the Directors of the Plessey Co. Ltd. for permission to publish this paper and Mr. P. Gurnell of Services Electronics Research Laboratory, Baldock, for supplying samples of standard silicon and for encouragement throughout this work.

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Discussion

Mr. J. F. Duke asked the authors to say what other elements they had determined by isotope dilution.

Mr. Newton replied that they had carried out some work on the determination of titanium in silicon, but this had not been completed. They were now trying to determine silicon in gallium arsenide by isotope-dilution analysis.

Dr. C. A. Parker congratulated the authors on their low blanks. He asked what precautions they had taken to achieve them.

Mr. Newton replied that the apparatus was housed in a pressurised laboratory, with a filtered air supply. All chemical manipulations were carried out in a form of dry box, and over-shoes were worn.

The Polarographic Determination of Niobium in Highly Alloyed Steels*

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A method is described for determining up to 3 per cent. of niobium in a wide range of ferrous alloys. The niobium is separated from the major alloying constituents as niobic acid, together with other earth-acid hydrated oxides, by hydrolysis in the presence of sulphurous acid.

The oxides are ignited and fused with potassium hydroxide, the melt is extracted with dilute potassium hydroxide solution, an aliquot of the solution is adjusted to pH 1·9 in the presence of EDTA and the polarographic wave of niobium is recorded directly. In the presence of molybdenum, a solvent extraction precedes the final polarographic determination.

Niobium has found increasing uses in recent years in austenitic stainless steels, as a stabiliser, and in certain high-temperature alloys. The niobium is invariably accompanied by some tantalum in these materials. For the purpose of metallurgical research it was desirable to know the precise niobium content of an alloy or steel independent of the tantalum content.

Tantalum *plus* niobium could be determined satisfactorily by Bagshawe and Elwell's method,[1] but, after a fairly extensive search through the various available methods for the individual determination of these elements,[2] there appeared to be room for further work.

Separation of niobium and tantalum by chromatography on a cellulose column, with fluoride solutions, is well known,[3,4,5] but a procedure based on this principle was considered too time-consuming. Further, the particular solvents are unpleasant to handle. Colorimetric methods for determining niobium and tantalum are available; for example, the thiocyanate complex of niobium forms the basis of several colorimetric procedures,[6,7,8] but these are limited in their application, particularly when a filter type instrument is used. Pyrogallol forms a yellow complex with niobium in alkaline solution and a similar complex is formed with tantalum in acid solution,[9] but consideration was not given to these methods, because of the interference due to titanium. Polarographic methods for the separate determination of niobium and tantalum were considered and attention was directed towards Ferrett and Milner's paper,[10] wherein the application of polarographic procedures in base solutions of sulphuric,[11] hydrochloric,[12,13] tartaric[14] and oxalic[14] acids is examined. Ferrett

*Presented at the meeting of the Midlands Section of the Society on Tuesday, September 13th, 1960. This paper received the Elwell Award for 1960.

and Milner have also reported on the polarographic steps obtained for niobium in other media, for example, malic, lactic, gluconic and citric acids, but in none of these contributions did the authors observe a wave due to tantalum.

More recently papers have appeared describing the polarographic determination of niobium in steels; for example, Vivarelli,[15] following earlier work by Cozzi and Vivarelli, recovered the earth-acid oxides, fused them in sodium carbonate, etc., then finally determined niobium with a saturated hydrochloric acid - sodium chloride base solution. This method was tried, but was not found to be convenient for routine laboratory use; for example, hydrogen chloride gas is required, saturated hydrochloric acid solutions caused corrosion of the thermostat bath and the high residual cell current proved troublesome, even when a high counter-current was used. Mukhina and Tikhonova[16] utilised the niobium wave due to the citrate complex in 10 *N* hydrochloric acid; their method was more convenient, but poor wave form and high residual currents were observed and the solutions were equally corrosive to the instrument.

The most promising solutions for further investigating the polarography of niobium that appeared to have received little or no attention were two described by Ferrett and Milner,[10] namely, 2 *M* citric acid and EDTA (ethylenediaminetetra-acetic acid) solutions of pH less than 6·0. The authors stated that with the citric acid base electrolyte satisfactory solutions could only be prepared starting from metallic niobium. This would severely limit the analytical applications of this electrolyte and render it unsuitable for the proposed method. Since Ferrett and Milner had used potassium niobate to prepare EDTA solutions of niobium, this medium seemed best suited for adaptation to an analytical method. It was proposed to separate the niobium as niobic acid (together with other earth acids) by a hydrolytic procedure, ignite the niobic acid to niobium pentoxide, and convert the oxide to potassium niobate; an EDTA solution of the extract could then be prepared for polarographic examination. Ferrett and Milner, who used conventional equipment, reported two polarographic waves for niobium in EDTA media, the first with heights proportional to niobium concentrations in the range 5 to 500 μg per ml. The half-wave potential of the first wave varied with pH; it was −0·427 volt at pH 1·1 and −0·835 volt at pH 4·75. The wave was reversible between pH 2·0 and 3·2, the criterion of reversibility being the value of the inverse slope of applied voltage, v, against $\log_{10} i/(i_d - i)$, and the authors quoted values for the slope at each pH value studied. A concentration of 0·1 *M* EDTA was used above pH 3·0; below this pH EDTA becomes decreasingly soluble and lower concentrations were used. The authors recommended that suitable solutions could be prepared by dissolving potassium niobate in 15 ml of *M* potassium hydroxide, adding 3·7 g of disodium EDTA and diluting to 90 ml. The pH is adjusted to the required value in the range 3 to 6 with 10 per cent. v/v sulphuric acid, and the solution is diluted to 100 ml.

The second wave for niobium in this medium coalesces with the hydrogen wave and is of no analytical value.

The interference of other elements with the niobium waves was not described by these workers.

Experimental

Preliminary work—

Weighed amounts of niobium pentoxide were fused separately with potassium carbonate, sodium carbonate and potassium hydroxide in attempts to prepare potassium niobate solutions suitable for subsequent polarography in the acidified EDTA medium. Poor results were obtained with carbonate fusions; wave heights for a given concentration of niobium were much smaller and the waves more poorly defined than when potassium hydroxide was used. Amounts of niobium pentoxide in the range 5 to 25 mg (equivalent to 3·5 to 17·5 mg of niobium or 0·35 to 1·75 per cent. of niobium in a 1-g sample) were weighed into platinum crucibles, a 4·0-g portion of potassium hydroxide was added to each, and the crucibles were heated gently in a small flame until clear melts were produced. The melts were cooled, and extracted with 80-ml portions of water containing 1·6 g of potassium hydroxide. The solutions were transferred to separate 100-ml calibrated flasks and diluted to 100 ml with water. These solutions were approximately *N* in potassium hydroxide and contained between 35 and 175 μg of niobium per ml. Solutions suitable for polarography were prepared from a 20-ml portion of each solution by adding 0·29 g of EDTA, acidifying to pH 3·1 with 10 per cent. sulphuric acid and diluting to 100 ml. These 0·01 *M* EDTA solutions contained between 7 and

35 μg of niobium per ml, which was in the lower range of concentrations covered by Ferrett and Milner's investigation. However, when polarograms of these solutions were recorded over the range −0·4 to −0·9 volt against the mercury-pool anode, it was found that step heights were not sufficiently linear for analytical purposes. If these solutions were left overnight and polarograms recorded next day, an increase in wave height of up to 30 per cent. occurred. A similar series of solutions was prepared and, after adjustment to pH 3·0, the solutions were boiled for increasing periods of time before dilution to 100 ml. It was found that more than 20 minutes' boiling promoted no further increase in wave height, but the heights of these waves were still not linear. In several of the solutions there appeared a pale yellow gelatinous precipitate, which was shown to be platinic hydroxide. Clearly, the platinum crucible was being attacked in the fusion and it was thought that some co-precipitation of niobium with the platinum might be causing the non-linearity of the wave heights.

This trouble was overcome by using a gold crucible. On acidification of EDTA solutions before boiling, any gold that had dissolved during fusion formed a colloidal sol, which, when boiled for 30 minutes, deposited the gold as a fine film on the surface of the solution.

Further attention was paid to the conditions of fusion. It was found that the best results were obtained when the melt was swirled continuously during the fusion to aid loss of moisture, prevent mechanical loss and allow access of air to the melt. If the temperature of the melt was raised too rapidly effervescence on the surface of the gold was observed. The optimum rate of heating was such that no bubbling occurred. In this manner the temperature was slowly raised to about 400° C in about 3 to 5 minutes. When fusions were carried out precisely in this manner, the resulting solutions gave waves for niobium, the heights of which showed excellent linearity.

By fusing the same weight of niobium pentoxide repeatedly under slightly different conditions and recording polarograms on the resulting solutions, several interesting points were noted. If too low a temperature or too short a time was used for the fusions, the first wave height was reduced and that of the second wave, which coalesced with the hydrogen wave, was increased. If the solutions were boiled for 30 minutes, there was an increase in the first wave height and an apparent reduction in the second step height. When fusions were performed under the precise conditions described above and the EDTA solutions boiled for 30 minutes before polarography, constant wave heights were obtained, which were proportional to the niobium concentrations in the range studied (7 to 35 μg per ml in the final 100 ml of solution).

It having been established that potassium niobate could be prepared quantitatively from niobium pentoxide and that from the niobate a niobium - EDTA complex suitable for quantitative polarography could be prepared, the interference of other elements was investigated.

Interference from other elements—

By using the proposed hydrolysis procedure to separate the earth-acid oxides from the major alloying components of the steel, only traces of the alloying elements should be present. The mixture of oxides obtained would contain in addition to niobium, tantalum, tungsten and silicon and some of the titanium (even in steels nominally free from the metal) and molybdenum. The possible interference of each of these elements was investigated separately.

For each, an amount of the oxide equivalent to 10 mg of the element was fused with potassium hydroxide in exactly the same way as the niobium pentoxide had been fused. After treatment of a portion of each of the resulting solutions with EDTA and adjustment of pH to between 1·5 and 4·0, polarograms were recorded.

Tantalum, tungsten and silicon produced no wave and did not interfere with the niobium wave.

A polarographic wave for titanium^{IV} in EDTA medium at pH 1·0 to 2·5 has been reported ($E_{\frac{1}{4}} = -0{\cdot}22$ volt against the saturated-calomel electrode).[17] It was found, however, that solutions of titanium prepared in the manner described above gave weak steps. Extraction of the melt with dilute potassium hydroxide solution caused much of the titanium to be precipitated, and a filtered portion was used for preparing the EDTA solution. In the pH range 1·5 to 4·0 the wave produced by titanium occurred at about −0·4 volt against the mercury-pool anode. A solution was prepared by fusing oxides equivalent to 10 mg of titanium and 10 mg of niobium, and subsequent polarographic examination showed that

the titanium wave preceded that of niobium in EDTA solution by about 0·25 volt, but did not interfere in the pH range studied.

Přibil and Blazek[18] reported a reduction wave for molybdate ions in EDTA solutions buffered with acetic acid and ammonium acetate ($E_{\frac{1}{4}} = -0{\cdot}63$ volt against the saturated-calomel electrode). A similar wave was observed in the pH range 1·5 to 4·0 in the medium being studied, and severe interference with the niobium wave resulted when solutions containing molybdenum and niobium together were examined. The molybdenum wave precedes that due to niobium and coalesces with it, making polarographic measurements impossible.

INTERFERENCE BY MOLYBDENUM—

It was desirable that some means of overcoming the interference by molybdenum should be found. Taylor[19] had shown that up to 0·5 mg of molybdenum^{VI} could be completely extracted from 0·0001 *M* solutions of EDTA over the pH range 1·4 to 5·0 by shaking with 1 per cent. solutions of 8-hydroxyquinoline in chloroform. It was suggested that only tungsten might interfere, but no specific mention was made of the behaviour of niobium under these conditions. The possibility of extracting with 8-hydroxyquinoline the molybdenum from the EDTA solution, leaving the niobium as the EDTA complex, was therefore investigated.

Solutions were prepared containing between 7 and 35 μg of niobium per ml in EDTA. Separate 25-ml portions were shaken with solutions of 8-hydroxyquinoline in chloroform of different concentrations, *e.g.*, 1, 0·5, 0·2 and 0·1 per cent., and polarograms were recorded before and after extraction. It was found that some suppression of the wave heights occurred in each instance, but not more than 30 per cent. suppression was produced after shaking for 2 minutes with two 10-ml portions of the 1 per cent. solution of 8-hydroxyquinoline. A wave due to reduction of 8-hydroxyquinoline occurred at about −1·0 volt, but did not interfere.

Mixtures of molybdenum and niobium were then investigated. A molybdenum content of 5 per cent. was considered a maximum in the types of alloys for which the proposed method was intended. It was known from previous work that up to 10 per cent. of the molybdenum present in a steel could be found in the mixed oxides after hydrolysis from dilute hydrochloric acid in the presence of sulphurous acid. Amounts of oxides containing 5 mg of molybdenum and between 3·5 and 17·5 mg of niobium (equivalent to a 1-g sample of a steel containing 5 per cent. of molybdenum and 0·35 to 1·75 per cent. of niobium) were fused by the prescribed method, and the extracts were diluted to 100 ml. Some 20-ml portions of these extracts were used to prepare a series of solutions in 100 ml of 0·01 *M* EDTA. These solutions were used to find the optimum conditions for the solvent extraction of molybdenum by 8-hydroxyquinoline. The pH was varied between 1·5 and 4·0 and various concentrations of 8-hydroxyquinoline in different solvents (chloroform, carbon tetrachloride and benzene) were tried; the time of shaking was varied. By recording polarograms before and after extraction it was possible to follow the progressive removal of molybdenum.

From this work it seemed that conditions were optimum when the pH was 1·9 to 2·0 and a 0·1 per cent. solution of 8-hydroxyquinoline in benzene was used. Two 10-ml portions shaken for 2 minutes each with 25 ml of the EDTA solutions effectively removed 0·25 mg of molybdenum. Under these conditions the niobium wave was suppressed by 15 per cent. of its height before solvent extraction. By adding 0·02 g of solid 8-hydroxyquinoline to an EDTA solution of niobium at pH 2·0, it was found that the niobium wave was suppressed by about 8 per cent. This suggested that the extraction of niobium by the benzene was much less than the 15 per cent. observed before and that much of the suppression of the niobium wave was due to modification of the supporting electrolyte and not to removal of niobium by extraction in the organic phase. It is appreciated, therefore, that the polarographic wave height is influenced by the amount of 8-hydroxyquinoline present and the extent to which niobium is lost in the organic extraction. However, these factors are relatively small and constant. Thus, by treating the sample and standard alike, wave heights are similar and bear a simple relationship to the niobium content and linear wave heights are obtainable.

After solvent extraction as described it was found that the heights of the modified niobium waves were directly proportional to the niobium contents of the solutions as prepared. Thus a means of suppressing interference by molybdenum had been found. Even traces of molybdenum were found to distort the beginning of the niobium wave; it was therefore necessary to incorporate solvent extraction in all determinations on steels.

Method

PROCEDURE—

Transfer 1 g of sample (for niobium contents between 0·5 and 1·5 per cent.) to a 400-ml beaker, add 50 ml of hydrochloric acid, sp.gr. 1·18, and heat to dissolve the metal. (Dropwise addition of concentrated nitric acid aids solution of certain types of alloys.) When reaction has ceased, add a slight excess of nitric acid, sp.gr. 1·42, dropwise to complete oxidation of the solution. Evaporate to dryness, and bake the residue lightly for a few minutes. To the residue add 30 ml of hydrochloric acid, sp.gr. 1·18, warm to dissolve the residue, and evaporate to about 15 ml, but ensure that no local drying out of metal salts occurs. Cool slightly, and stir while adding 150 ml of sulphurous acid (saturated solution of sulphur dioxide). Heat the solution to its boiling-point, and boil briskly for 2 to 3 minutes. Dilute the solution to about 200 ml with water, add a little crumbled ashless tablet or paper pulp, and again allow to boil for 2 to 3 minutes. Set the solution aside for a minimum of 30 minutes, although a longer settling time, say overnight, is permissible and may have advantages when only a small weight of earth oxides is present. Filter the solution through a Whatman No. 504 filter-paper to which has been added a little paper pulp, and transfer the precipitate quantitatively to the paper. Wash the paper and precipitate thoroughly with warm dilute hydrochloric acid (1 + 19). Transfer the paper to a gold crucible, dry, heat gently at first to destroy the organic matter, and finally ignite at 800° C for a few minutes, and then cool.

To the ignited oxides add 4 g of potassium hydroxide pellets, and support the crucible on a tripod with a pipeclay triangle. Play a small flame on the bottom of the crucible, allow the pellets to melt slowly, and then swirl the contents of the crucible gently by agitating the supporting triangle. Gradually raise the temperature at a rate such that effervescence from the gold surface is avoided. (The final temperature is about 350° to 400° C and is indicated by a slight refluxing of the melt on the sides of the crucible when agitation is momentarily ceased. The melt will become green due to traces of iron and chromium. The fusion should take at least 3 minutes to complete and longer if an appreciable amount of titanium dioxide is present.)

Cool the melt slowly, and extract with 80 ml of distilled water containing 1·6 g of potassium hydroxide. Agitate during solution of the melt with a polyethylene-covered rod (this prevents scratching the surface of the gold crucible). Transfer the solution to a 100-ml calibrated flask, and dilute to the mark.

Transfer a 20-ml aliquot, filtered if necessary (*e.g.*, if titanium is present), to a 150-ml beaker, and add 0·29 g of EDTA and 70 ml of water. With use of a pH meter, adjust the solution to pH 1·9 with dilute sulphuric acid (1 + 9). Add a few granules or beads to prevent bumping, and boil gently for 30 minutes; avoid excessive evaporation. Cool the solution, transfer to a 100-ml calibrated flask, and dilute to the mark with distilled water.

Transfer a 25-ml portion of this solution to a 100-ml separating funnel, add 10 ml of 0·1 per cent. w/v 8-hydroxyquinoline in benzene, and shake for exactly 2 minutes. Allow the layers to separate, run the lower aqueous layer into a second dry separating funnel, and repeat the extraction with a further 10 ml of 8-hydroxyquinoline solution for 2 minutes. Allow to settle, and transfer some of the lower aqueous phase through a dry filter-paper into a polarographic cell. Bubble nitrogen through the cell solution to remove dissolved oxygen, record a polarogram between −0·4 and −0·9 volt, and measure the height of the niobium wave ($E_{\frac{1}{4}} = -0{\cdot}65$ volt against the mercury-pool anode). Prepare a standard solution containing the equivalent of about 1 per cent. of niobium in a 1-g sample by weighing accurately about 14 mg of pure niobium pentoxide into a gold crucible and fusing with potassium hydroxide in exactly the same manner as with the mixed oxides from a sample. Dissolve the melt in the same manner, and carry out the identical procedure as for the sample solution on a 20-ml portion of the extract.

Calculate the niobium content of the sample by comparing the niobium wave heights produced by the sample and standard.

Results

To indicate the precision of the proposed procedure the niobium contents of four British Chemical Standard alloys were determined. The compositions of the alloys and the results obtained are shown in Table I. Most of the certified determinations of niobium were carried out by Bagshawe and Elwell's method, which gives a combined value for niobium and tantalum.

A few analysts separated the two elements, and their results are shown in Table I. The proposed procedure covers a range of niobium contents between 0·5 and 1·5 per cent. approximately and molybdenum contents up to 5 per cent. These ranges could be extended in either direction by altering the weight of sample or the aliquot of the fusion extract taken.

A Cambridge pen recording polarograph was used for the development of the method.

TABLE I

DETERMINATION OF NIOBIUM

Alloy	Elements present	Concentration of element present, %	Range of certified niobium and tantalum contents, %	Niobium found by proposed method, %
Stainless steels—				
B.C.S. No. 246 ..	C	0·262	0·78 to 0·84 (one analyst reported <0·02% of tantalum)	0·75, 0·79, 0·76,* 0·76, 0·78
	Mo	2·89		
	Cr	18·8		
	Ni	12·1		
	W	0·22		
	Cu	0·13		
	Sn	0·01		
B.C.S. No. 261 ..	C	0·083	0·68 to 0·74 (one analyst reported 0·67% of niobium and 0·04% of tantalum)	0·66,* 0·67, 0·65, 0·66(5)
	Si	0·39		
	Cr	17·2		
	Ni	13·08		
	Mn	0·66		
Permanent-magnet alloy—				
B.C.S. No. 266 ..	Co	23·4	1·17 to 1·24 (no separate value given for tantalum)	1·17,* 1·12, 1·14
	Ni	13·3		
	Al	7·95		
	Cu	3·33		
	Ti	0·01		
	C	0·07		
	Si	0·22		
	S	0·04		
	Mn	0·27		
	Fe	49·7		
Mild steel—				
B.C.S. No. 271 ..	Ni	0·01	Niobium†—0·110 Tantalum—0·008	0·11,‡ 0·12,‡ 0·11‡
	Cr	0·04		
	Mo	0·19		
	Cu	0·01		
	W	0·02		
	Co	0·01		
	Sn	0·11		
	V	0·005		
	B	0·013		
	Si	0·32		
	Mn	0·46		
	Ti	0·005		
	Zr	0·05		

* Titanium, zirconium and vanadium (10 mg of each) added to sample during solution.
† Certified niobium content determined spectrographically was 0·13 per cent.
‡ Determined on 5-g sample.

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The Microbiological Assay of Thiamine

Two Methods Modified for use with Small Amounts of Test Material

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Two methods of thiamine assay, that of Fitzgerald and Hughes, in which *Lactobacillus fermenti* 36 is used, and that of Deibel, Evans and Niven, with *L. viridescens*, have been modified for use with small amounts of test material. The assays are carried out in 2-ml amounts in colorimeter tubes, and levels of thiamine down to 2 mμg can be determined.

Deibel, Evans and Niven's observation that *L. viridescens* is less susceptible than *L. fermenti* to inhibitory or stimulatory substances other than thiamine has been confirmed. Hence *L. viridescens* may be preferred to *L. fermenti* for thiamine assay.

THE microbiological methods recommended for the assay of thiamine by the Thiamine (Microbiological) Panel of the Sub-Committee on Vitamin Estimation of the Analytical Methods Committee[1] are the tube method of Fitzgerald and Hughes[2] and the plate method of Jones and Morris.[3] In both methods use is made of *Lactobacillus fermenti* 36. A method in which *L. viridescens* is used as assay organism has been described recently by Deibel, Evans and Niven.[4] The thiochrome method of the Analytical Methods Committee,[5] however, is the preferred procedure for thiamine assay.

During some experiments on rat nutrition we wished to determine thiamine in small samples of faeces (about 0·1 g) containing sometimes as little as 1 μg of thiamine per g, and we found it necessary to use a microbiological method, since the thiochrome method proved, with the apparatus available, to be insufficiently sensitive. However, the plate method of Jones and Morris[3] is not suitable for materials containing less than 10 μg of thiamine per g,[1] and we found that for the tube methods described by Fitzgerald and Hughes[2] and by Deibel, Evans and Niven[4] the samples available provided insufficient material. This paper describes Fitzgerald and Hughes' tube method as modified for our work to deal with small amounts of test material and also the method of Diebel *et al.* similarly modified. Although the modifications to the original methods are not great, we thought it necessary to carry out a series of experiments to establish the accuracy of the new methods, since changes in conditions such as the ratio of surface to volume might well affect the assay.

Results of assays with *L. viridescens* have been compared with those obtained with *L. fermenti* and also with results of assays by the yeast-fermentation method of Atkin, Schultz and Frey.[6] The effects on the two lactobacilli of interfering substances in the test materials have been compared by calculating results for each organism on the basis of two different sets of standards: (*a*) normal standards containing only thiamine solution and (*b*)

standards containing, in addition to the thiamine solution, test material in which thiamine had been destroyed by sulphite, as recommended by Fitzgerald and Hughes for *L. fermenti*.

Methods

The following is a description of the modified methods for the two thiamine assays as they were finally carried out after a series of trial experiments. Methods for the two organisms are similar in many respects and are therefore dealt with together, differences between the two being noted.

Organisms—

The organisms used were *L. fermenti* 36 and *L. viridescens** strain S38A (ATCC No. 12706).

Stock cultures—

These were maintained as stab cultures on the media described below—

Lactobacillus fermenti—Glucose, 1 per cent.; tryptone, 1 per cent.; K_2HPO_4, 0·2 per cent.; yeast extract, 0·2 per cent.; Oxoid dehydrated liver infusion, 0·2 per cent.; sodium acetate, 1 per cent.; agar, 1·5 per cent.; thiamine, 1 μg per ml; pH 6·8. This medium was based on a formula recommended by Mr. N. J. Butler (personal communication).

Lactobacillus viridescens—APT broth, single strength (medium of Deibel *et al.* obtained as Difco "Bacto" dehydrated medium No. 0808) *plus* 1·5 per cent. of agar and 1 μg of thiamine per ml.

The media were made up freshly at intervals of not more than 3 weeks. Cultures were incubated for 24 to 48 hours, *L. fermenti* at 37° C and *L. viridescens* at 30° C, and stored at refrigeration temperature (about 4° C). Subcultures were made at fortnightly intervals.

Inoculum for assay—

The media described below inoculated directly from stock cultures were used for preparation of inocula—

Lactobacillus fermenti—Micro-Inoculum broth (Difco dehydrated medium) *plus* 1 μg of thiamine per ml.

Lactobacillus viridescens—APT broth at single strength *plus* 1 μg of thiamine per ml.

Cultures were incubated at 37° and 30° C, respectively, for 22 to 26 hours. They were spun in a centrifuge aseptically, washed twice in sterile saline, re-suspended in 10 ml of saline and diluted 1 in 10 or to give a faintly turbid suspension. One drop from a Pasteur pipette was used for inoculation.

Assay medium—

Lactobacillus fermenti—Difco "Bacto" dehydrated thiamine assay medium (B 326).
Lactobacillus viridescens—Difco "Bacto" dehydrated thiamine assay medium (0808).
Double-strength medium was prepared freshly for each assay.

Thiamine solutions—

Stock solution—This was made up at a concentration of 1000 μg per ml in dilute acid solution, 0·25 g of thiamine being dissolved in a small amount of distilled water and made up to 250 ml with 2 per cent. hydrochloric acid. This solution was stored at refrigeration temperature under sulphur-free toluene and was renewed at 3-monthly intervals.

Working standard—Immediately before use, stock thiamine solution was diluted with 0·01 *M* phosphate buffer to give a solution containing 20 mμg of thiamine per ml.[1]

Extraction methods—

In the series of test assays two types of test material were used, namely, dried yeast and faeces or caecal contents of rats on experimental diets.

For the dried yeast, digestion and extraction were carried out by methods similar to those of Fitzgerald and Hughes,[2] but suitably adjusted for small quantities. Two extracts were prepared—Extract I, in which thiamine was to be determined, and Extract II, which

* The strain of *L. viridescens* used in these experiments was kindly provided by Dr. C. F. Niven, jun., of the American Meat Institute Foundation, Chicago.

had been treated with sulphite in order to destroy thiamine. The latter extract was then added to standard tubes, as recommended by Fitzgerald and Hughes, to allow for any effect of inhibitory or stimulatory substances other than thiamine. In neutralising the sulphite with hydrogen peroxide we found it necessary to complete the neutralisation with 2·5-volume hydrogen peroxide in place of the 10-volume peroxide used by Fitzgerald and Hughes. In particular, *L. viridescens* was found to be sensitive to minute amounts of hydrogen peroxide or of sulphite.

For assays of rat faeces or caecal contents, when we were interested only in free thiamine, no digestion was carried out. The sample was extracted in 0·1 *N* sulphuric acid at room temperature for 30 minutes, being shaken at intervals. Extracts I and II were then prepared as for the dried yeast.

Extracts were made up to volume to contain from 20 to 40 mμg of thiamine per ml.

ASSAY PROCEDURE—

The assay was set up in 8-mm × 80-mm E.E.L. colorimeter tubes (Evans Electroselenium Ltd.), the total volume in each tube being 2 ml. The use of this smaller volume facilitated the assay of smaller amounts of test material and also rendered the assay sensitive to lower thiamine levels. Use of colorimeter tubes minimised the work involved in reading the assay. The glassware could also more readily be kept separate from that used for other purposes.

The pattern of the assay for the preparation of standard curves in this series of experiments was similar to that established by Fitzgerald and Hughes. The extract of test material (Test Extract I) was assayed at three concentration levels. Four series of standard tubes were prepared, each containing standard solution at levels of 0, 4, 6 or 8 mμg of thiamine per tube. To three of these series of standard tubes Test Extract II was added at the three concentrations used for Test Extract I in preparation of the test curve. Uninoculated blanks were included in all experiments, and all tubes were set up in triplicate.

It was found unnecessary to mix the contents of the assay tubes at this stage, provided that the 1 ml of assay medium was added last.

Tubes were plugged with cotton-wool and heated in an autoclave for a brief period, the pressure being brought up to 10 lb per sq. inch and the autoclave then turned off immediately. A small autoclave was used to avoid overheating. Tubes were cooled in the dark, inoculated and tapped to mix the contents.

Incubation was at 37° C for *L. fermenti* and 30° C for *L. viridescens*, each for 17 hours. The tubes were then placed in the refrigerator for 15 minutes, and turbidities were read with an E.E.L. photoelectric colorimeter.

GLASSWARE—

All glassware was prepared by soaking it in 6 per cent. chromic acid for at least 2 hours and then washing it six times in tap-water and twice in distilled water. This is not in accordance with the more elaborate method recommended by the Analytical Methods Committee,[1] but standard curves prepared for both *L. fermenti* and *L. viridescens* with glassware prepared by the recommended method and by our method showed no significant difference.

RESULTS

The accuracy of the methods described above was checked in the following series of assays.

STANDARD CURVES—

Standard curves (Figs. 1 and 2) obtained in the small-tube method are linear or nearly so for both organisms, slight day-to-day variation occurring. The effective assay range for *L. fermenti* is from 2 to 8 mμg of thiamine per tube, an increase in response of approximately 10 colorimeter units occurring over this range; for *L. viridescens*, the range is from 2 to at least 15 mμg of thiamine per tube, the increase in response of this organism over the same range of thiamine concentrations being slightly less than that of *L. fermenti*.

The depressed dose - response curves noted by many workers with *L. fermenti* were found occasionally in this work with each organism, although less frequently with *L. viridescens*. This was sometimes due to the batch of dehydrated assay medium used; on other occasions no cause was found. All such assays were discarded.

Assays carried out in large tubes containing the same amount of thiamine per ml as the small tubes gave similar dose - response curves.

COMPARISON OF THIAMINE VALUES OBTAINED WITH *L. fermenti* AND *L. viridescens* FOR DRIED YEAST: RECOVERY TESTS—

Tables I and II show thiamine values and recovery-test results, for the same sample of dried yeast, obtained with *L. fermenti* and *L. viridescens*. The assays were carried out at intervals over 2 months. Results calculated on the basis of standards with and without sulphite-treated extracts are shown.

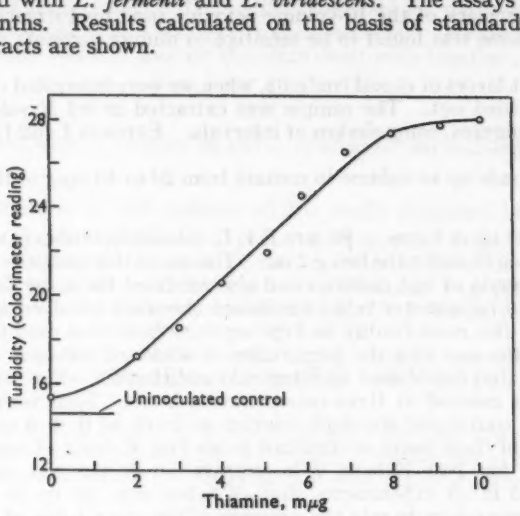

Fig. 1. Standard curve for small-tube (2 ml) assay of thiamine with *L. fermenti* 36

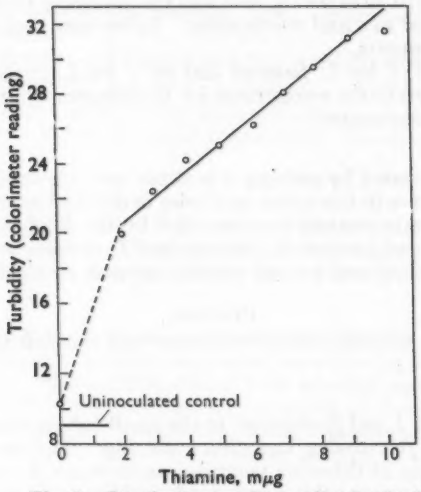

Fig. 2. Standard curve for small-tube (2 ml) assay of thiamine with *L. viridescens*

Comparison of the figures obtained from the yeast sample, calculated on the basis of standards containing sulphite-treated extracts, the method recommended by Fitzgerald and Hughes for *L. fermenti*, shows good agreement between the two organisms, *L. fermenti* giving a mean value of 0·955 μg of thiamine per g and *L. viridescens* a mean of 0·98 μg of thiamine per g. Statistical comparison of these two sets of results shows no significant difference at the 25 per cent. level between the two mean values for thiamine obtained, t being 0·46 for

11 degrees of freedom. Standard deviations were 0·13 and 0·06, respectively. Comparison of variances for the same set of figures gave, for degrees of freedom $\nu_1 = 5$, $\nu_2 = 6$, an F value of 5·62. Its significance thus lies between the 5 and 10 per cent. levels.

From evidence provided by this analysis it appears, therefore, that the two lactobacillus methods of assay are likely to give similar results when calculation is based on standards containing sulphite-treated extracts. The *L. viridescens* method may be expected to show slightly less variation than *L. fermenti*, although not significantly so.

Recovery tests were in general acceptable, but tended to be rather low for *L. viridescens*.

TABLE I

RESULTS OF THIAMINE ASSAYS AND RECOVERY TESTS WITH *L. fermenti* 36 ON A SAMPLE OF DRIED YEAST

Results calculated on basis of standards containing sulphite-treated extract			Results calculated on basis of standards without sulphite-treated extract		
Thiamine found in—			Thiamine found in—		
dried yeast, μg per g	dried yeast *plus* 4 μg of thiamine per g, μg per g	Recovery of added thiamine, %	dried yeast, μg per g	dried yeast *plus* 4 μg of thiamine per g, μg per g	Recovery of added thiamine, %
Assay results—					
1·18	5·5	108	1·80	5·9	102
1·02	—	—	1·57	—	—
0·90	4·5	90	1·08	5·1	100
0·80	5·3	112	1·20	5·8	116
0·92	5·4	112	0·97	5·5	113
0·91	4·8	98	1·00	4·7	92
Mean value—					
0·955	5·1		1·27	5·4	
Range of variation from mean, %—					
−15·8 to +24·2	−11·8 to +7·8		−23·6 to +41·7	−12·96 to +9·3	
Standard deviation—					
0·13	0·43		0·34	0·5	

TABLE II

RESULTS OF THIAMINE ASSAYS AND RECOVERY TESTS WITH *L. viridescens* ON A SAMPLE OF DRIED YEAST

Results calculated on basis of standards containing sulphite-treated extract			Results calculated on basis of standards without sulphite-treated extract		
Thiamine found in—			Thiamine found in—		
dried yeast, μg per g	dried yeast *plus* 4 μg of thiamine per g, μg per g	Recovery of added thiamine, %	dried yeast, μg per g	dried yeast *plus* 4 μg of thiamine per g, μg per g	Recovery of added thiamine, %
Assay results—					
1·00	—	—	1·07	—	—
1·08	4·2	77	1·22	3·8	65
0·93	4·9	99	1·04	5·2	105
0·96	4·2	80	0·99	4·8	97
0·92	4·6	92	1·04	4·5	87
0·98	6·0	126	0·94	4·8	96
1·02	4·9	97	0·93	4·5	89
Mean value—					
0·98	4·8		1·03	4·6	
Range of variation from mean, %—					
−6·1 to +10·2	−12·5 to +25·0		−9·7 to +18·4	−17·4 to +13·0	
Standard deviation—					
0·06	0·67		0·10	0·49	

REPRODUCIBILITY OF RESULTS—

Assays of the same sample of dried yeast carried out over 2 months (Tables I and II) show with both *L. fermenti* and *L. viridescens* good reproducibility of results.

COMPARISON OF THIAMINE VALUES OBTAINED WITH THE TWO LACTOBACILLUS METHODS AND THE YEAST-FERMENTATION METHOD FOR RAT FAECES AND CAECAL CONTENTS: RECOVERY TESTS—

Table III shows the results of a series of experiments carried out over 6 weeks in which thiamine was determined in rat faeces or caecal contents with *L. fermenti* or *L. viridescens* and by the yeast-fermentation method.

TABLE III

RESULTS OF THIAMINE ASSAYS AND RECOVERY TESTS BY PROPOSED AND YEAST-FERMENTATION METHODS ON SAMPLES OF RAT FAECES OR CAECAL MATERIAL

	Results calculated on basis of standards containing sulphite-treated extract					Results calculated on basis of standards without sulphite-treated extract	
	Thiamine found in sample by—		Recovery of added thiamine			Thiamine found in sample by proposed method,	Recovery of added thiamine by proposed method,
Sample No.	proposed method, μg per g	yeast method, μg per g	Thiamine added, μg per g	Recovery by proposed method, %	Recovery by yeast method, %	μg per g	%
L. fermenti 36 *used in proposed method*—							
1	5·3	4·4	16·1	99	94	5·7	101
2	5·3	4·6	16·1	116	89	6·4	123
3	4·8	5·3	16·0	116	98	5·4	122
4	2·8	2·9	4·0	60	88	2·5	98
L. viridescens *used in proposed method*—							
5	5·6	5·0	14·3	101	98	5·9	90
6	4·4	5·0	15·3	85	85	3·9	84
7	6·3	5·6	15·8	69	66	6·4	68
8	3·9	4·0	16·1	133	100	3·7	128
9	1·3	1·3	4·0	119	90	1·5	133

The yeast and lactobacillus methods (calculated on the basis of standards containing sulphite-treated extract) show reasonable agreement, although recovery tests are rather less satisfactory than for the assays of dried yeast. No explanation has been found for this.

COMPARISON OF VALUES CALCULATED ON THE BASIS OF STANDARDS WITH AND WITHOUT SULPHITE-TREATED EXTRACTS—

Values obtained with *L. fermenti* and *L. viridescens* and each type of standard are compared in Tables I and II for a dried-yeast sample and in Table III for rat faeces and caecal contents. The thiamine level in faeces and caecal samples was variable, but in the assays of yeast should not vary, as the same dried-yeast sample was used throughout. Comparison of the two means (0·955 and 1·27) obtained with *L. fermenti* for dried yeast (see Table I) gave t to be 2·12 for 10 degrees of freedom. This is almost significant at the 5 per cent. level, the t value for which is 2·23. Comparison of variances of the two sets of figures obtained by calculating thiamine values from standards with and without sulphite-treated extracts gave an F value of 6·75 ($\nu_1 = 5$, $\nu_2 = 5$), which is again almost significant at the 5 per cent. level. Comparison of the two means (0·98 and 1·03) obtained with *L. viridescens* (see Table II) gave $t = 1·18$ for 12 degrees of freedom. There is thus no significant difference between these two means at the 25 per cent. level. A comparison of variances for the *L. viridescens* figures gave an F value of 3·12 ($\nu_1 = 6$, $\nu_2 = 6$). This is not significant at the 10 per cent. level.

Thus, neither with *L. fermenti* nor *L. viridescens* do results calculated on the basis of standards containing sulphite-treated extracts differ significantly from those calculated on the basis of normal standards. With *L. fermenti*, however, the results differ sufficiently (the difference being almost significant at the 5 per cent. level) to make desirable the use of sulphite-treated extracts, whereas with *L. viridescens* much less variation may be expected, rendering unnecessary in this assay the use of such extracts.

Comparison of results from assay of faeces and caecal material confirms this observation.

Discussion of the Assays

The microbiological assay procedures for thiamine recommended by the Analytical Methods Committee require relatively large amounts of the test substance and of other materials. The assays described here are more economical of materials and incubator space, are simple to operate, being carried out directly in colorimeter tubes, and can be used to determine thiamine down to a level of 2 mμg. Both *L. fermenti* and *L. viridescens* methods as described gave satisfactory results, assays with the two organisms giving thiamine values in agreement with each other and with those found by the yeast-fermentation method.

The finding of Deibel *et al.*[4] that *L. viridescens* is less sensitive to inhibitory and stimulatory substances other than thiamine has been confirmed by demonstrating no significant difference in results calculated on the basis of standard curves with and without sulphite-treated extracts of test substances (in this work limited to dried yeast and faeces or caecal contents of rats). The *L. fermenti* method, on the other hand, still has the disadvantage inherent in the use of this organism, namely, that it responds to substances other than thiamine, thus making it necessary to prepare sulphite-treated extracts of test substance as described by Fitzgerald and Hughes. Hence the design of the *L. viridescens* assay can be simpler than that of the *L. fermenti* assay and the number of tubes fewer, since standards containing sulphite-treated extracts are unnecessary and can be omitted in routine assay with the former organism. In such assays at least five concentrations of test extract should be set up to establish a test curve.

Results of this work support the suggestion of Deibel *et al.* that the *L. viridescens* method may prove to be the more convenient of the lactobacillus methods for thiamine assay.

I wish to thank Dr. H. R. B. White for her continued help and encouragement during this work and Mrs. V. M. Hedley for her collaboration in carrying out the yeast-fermentation assays and for permission to publish her results. I also thank Mr. E. E. Fitzgerald and Professor J. Yudkin for valuable criticism of the manuscript. Finally I express my appreciation to Mr. F. Bush and Miss C. Pretious for their excellent technical assistance.

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Colorimetric Micro-determination of Calcium in Serum

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Calcium is precipitated directly, as its naphthalhydroxamate, by adding a slight excess of precipitant to a 0·2-ml portion of serum. After centrifugation, the excess of reagent is removed by decantation and drainage, and the unwashed precipitate is dissolved in alkaline ethylenediaminetetra-acetate solution. Addition of acid ferric nitrate develops an orange-red colour, the intensity of which is a measure of the amount of calcium originally present in the serum.

The standard method[1] for determining calcium in serum involves direct precipitation of the insoluble oxalate, which, after washing, is dissolved in acid and titrated with standard permanganate or another suitable oxidant. To achieve the requisite sensitivity, 2 ml of serum are required for analysis, and a titre of about 1 ml of 0·01 *N* permanganate is obtained when the serum contains 10 mg of calcium per 100 ml, the amount present in normal serum. When other determinations may be required on the same sample of serum or when the specimen is

from a child, 2 ml is too great a volume to use, and efforts have been made to reduce the amount required for determining calcium. Owing to the appreciable solubility of calcium oxalate, it is undesirable to use smaller amounts of serum in the standard method, but, by washing the precipitate with ammonium oxalate, thus introducing a common ion and decreasing the solubility of calcium oxalate, it is possible quantitatively to isolate the calcium from small volumes of serum. Unfortunately, the only suitable finish to such a determination involves ashing the precipitate to the oxide or carbonate and subsequent acid - alkali titration, which is unsatisfactory with the very small amounts of alkali present.

Various other precipitants have been used for the micro-determination of serum calcium, but few have any advantage over oxalate. The two most frequently used have been picrolonic acid and chloranilic acid, but the solubility of the calcium salts of both these acids is too great to permit accurate determinations by direct precipitation methods on 0·2 ml or less of serum.

In the past 15 years, a different approach to the problem has been made by titrating the calcium with ethylenediaminetetra-acetic acid (EDTA), which forms a chelate compound with calcium in alkaline solution. With calcium-specific dyes as indicators, numerous methods involving 0·2 ml or less of serum have been reported, but the end-point is difficult to observe at the high dilutions used and photometric observation of the end-point is necessary if a satisfactory finish is to be obtained. Many photometers are not suitable for this type of titration, and a special instrument fitted with a magnetic stirrer must be used.

Another method of attack is to determine the calcium by means of its coloured complexes with various dyes. These methods are highly sensitive; they suffer from the disadvantage that the initial optical density of the dye solution is high compared with the increase in optical density produced by the presence of calcium, thereby reducing the accuracy of the readings. Another difficulty with both the chelation and direct-colour methods is that the blank value for the solutions used is relatively high, and specially purified water and reagents must be used.

Since a relatively inexpensive flame photometer came on the market about 10 years ago, largely for the determination of sodium and potassium, attempts have been made to use the instrument for the direct determination of serum calcium, but, owing to the presence of interfering substances, it is necessary first to isolate the calcium. Even so, two of the earlier methods required 2 ml of serum[2,3]; Butterworth's method[4] requires only 0·2 ml of serum, but this is achieved by accepting a low standard of sensitivity, and the photometer readings obtained by this method are low. MacIntyre[5] described a flame-photometric method involving direct spraying of a protein-free serum extract. The method appears to have high sensitivity; its disadvantage is that it requires the use of an oxygen - acetylene flame spectrophotometer of special design, and it is unlikely that such an instrument will be much used in routine hospital laboratories.

What is wanted in such a laboratory is a method that works well with 0·2-ml amounts of serum, is simple and accurate and involves use of the ordinary apparatus normally found in small departments. A suitable precipitation method would fulfil these requirements; further, it would probably be possible to free the reagents from calcium, thereby decreasing the blank value to negligible proportions. The experiments described below were conducted with the object of devising such a method.

Experimental

Several possible precipitants were examined, including ferrocyanide, picrolonic acid and chloranilic acid, but the calcium salts of these acids were all too soluble to permit accurate determinations on 0·2-ml amounts of serum. Eventually, naphthalhydroxamic acid, the reagent described by Beck,[6] was tested and showed promise of fulfilling the required conditions. Naphthalhydroxamic acid has the formula $C_{10}H_6(CO)_2NOH$; it is a pale yellow powder and is insoluble in water, but soluble in alkalis to form a yellow solution. The calcium salt is insoluble and is formed from pure solutions of calcium as a finely divided precipitate, difficult to separate by centrifugation. However, it was observed that, in the presence of sodium chloride and other salts, a crystalline precipitate was formed that was easy to separate. Such a reagent would precipitate 20 μg of calcium from 5 ml of solution at any pH from 8 to 14 when only a small excess of precipitant was used, and it was possible to dispense with washing of the deposit before colour development. The naphthalhydroxamic acid in the calcium salt can be determined colorimetrically, as the yellow solution in alkalis, but the colour gives an optical density of only 0·2 when 20 μg of calcium in a final volume of 4 ml are used and

measurements are made in a cell of 10-mm light-path with the most sensitive available filter (Chance OB10). However, as might be expected, naphthalhydroxamic acid gives a colour with ferric salts; this has an optical density of 0·4 under the above conditions, and a method based on direct precipitation of calcium from 0·2 ml of serum was devised involving use of this colour reaction.

METHOD

GLASSWARE—

Cylindrical centrifuge tubes of capacity 12 to 15 ml. Clean the tubes by filling them with chromic acid solution (10 per cent. w/v of chromium trioxide and 20 per cent. v/v of sulphuric acid) and setting them aside overnight or longer. Just before use, rinse the tubes with water until all traces of yellow are removed. Pour off as much of the wash water as possible, and use the tubes wet.

REAGENTS—

Use only reagents of recognised analytical grade, and store all reagent solutions in plastic bottles. The solutions can be stored for at least 6 months.

Water—Ordinary distilled water is suitable for use, as any calcium present is removed in preparing the precipitant. If water obtained by passing distilled water through a mixed ion-exchange resin is available, it is naturally to be used in preference.

Calcium reagent solution—With the aid of heat, dissolve 250 mg of naphthalhydroxamic acid, $C_{10}H_6(CO)_2NOH$ (obtainable from Hopkin & Williams Ltd., Freshwater Road, Chadwell Heath, Essex), in 100 ml of water containing 5 ml of ethanolamine and 2 g of D(+)-tartaric acid. Cool the solution, add 9 g of sodium chloride dissolved in 500 ml of water, and dilute to 1 litre with water. If a precipitate forms, filter the solution through an ashless filter-paper (Whatman No. 40, 41 or 43).

Alkaline EDTA solution—Dissolve 2 g of disodium ethylenediaminetetra-acetate dihydrate in 1 litre of 0·1 *N* sodium hydroxide.

Colour reagent solution—Dissolve 60 g of ferric nitrate, $Fe(NO_3)_3.9H_2O$, in 500 ml of water, add 15 ml of concentrated nitric acid, and dilute to 1 litre with water.

Standard calcium solution—Dissolve 100·1 mg of dry calcium carbonate in 30 ml of 0·1 *N* hydrochloric acid, and dilute to 200 ml with water. This solution contains 20 mg of calcium per 100 ml; for use, dilute with water to make appropriate standards.

PROCEDURE—

By means of a calibrated delivery pipette, place 0·2 ml of serum in a cylindrical 12- to 15-ml centrifuge tube. Add 5 ml of calcium reagent solution, set aside at room temperature for 30 minutes, and then spin in a centrifuge at 3500 r.p.m. and 6 inches radius for 5 minutes. Remove the clear supernatant liquid by inverting the tube and allowing it to drain vertically on a pad of filter-paper for 5 minutes. Wipe the mouth of the tube with an absorbent cloth, add 1 ml of alkaline EDTA solution, and shake to suspend the precipitate. Cover the mouth of the tube with an aluminium cap, and dissolve the precipitate by heating for 10 minutes in a bath of boiling water; shake the tube at intervals during the heating to ensure complete solution of the precipitate. When cool, add 3 ml of colour reagent solution, and mix the contents of the tube. Prepare a blank by carrying out the above procedure with omission of the serum. Set a photoelectric absorptiometer to zero with the blank, and measure the optical density of the test solution in 10-mm cells; use a Chance OB10 filter. (If a spectrophotometer is used, measure the optical density at 450 mμ). Calculate the calcium content of the serum by comparing the readings with those found for standard calcium solutions analysed together with the test. The optical densities found for solutions containing from 5 to 17·5 mg of calcium per 100 ml are between 0·2 and 0·8, the range in which the relative photometric error is least.[7] The colour deviates only slightly from Beer's law and the optical density does not alter for at least 2 hours after colour formation.

RESULTS

Portions (0·2-ml) of standard solutions of calcium were analysed by the proposed method, optical-density measurements being made in 10-mm cells with an absorptiometer (Chance OB10 filter). The final volume of coloured solution was 4 ml; the results were—

Calcium present, μg per 0·2 ml ..	0	5	10	15	20	25	30	35	40
Calcium present, mg per 100 ml ..	0	2·5	5	7·5	10	12·5	15	17·5	20
Optical density	0·0	0·1175	0·22	0·33	0·44	0·555	0·66	0·765	0·87

Recovery experiments were carried out on serum from which all the calcium had been removed by treatment with a mixed ion-exchange resin. The results in Table I indicate that recovery was quantitative over the range 5 to 15 mg of calcium per 100 ml of serum and was unaffected by the simultaneous presence of abnormal amounts of substances that could be expected to occur in serum. The figures in Table I refer to substances simultaneously added to serum; the same amounts of these substances separately added gave recoveries in the range 98 to 102 per cent. when the serum contained 10 mg of calcium per 100 ml. In particular, the maximum permissible concentration of magnesium sulphate heptahydrate was 100 mg per 100 ml, *i.e.*, 10 mg of magnesium per 100 ml. This figure is unlikely to be exceeded in serum.

TABLE I

RECOVERY OF CALCIUM ADDED TO CALCIUM-FREE SERUM

In absence of other substances			In presence of added substances*		
Calcium added, mg per 100 ml	Calcium found, mg per 100 ml	Recovery, %	Calcium added, mg per 100 ml	Calcium found, mg per 100 ml	Recovery, %
5	5·0	100	5	5·15	103
10	10·0, 10·2	100, 102	10	10·2	102
15	15·0	100	15	15·1	101

* The serum also contained, per 100 ml, 1000 mg of sodium chloride, 500 mg each of urea, glucose and sodium hydrogen carbonate, 250 mg of haemoglobin, 100 mg each of ammonium sulphate, potassium dihydrogen orthophosphate and $MgSO_4.7H_2O$, 25 mg each of heparin and bilirubin and 2 mg each of $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$ and $Fe(NO_3)_3.9H_2O$; these substances were simultaneously added to the serum

TABLE II

COMPARISON OF RESULTS FOR SERUM CALCIUM

	Calcium found by proposed method—				Calcium found by proposed method—		
Specimen No.	directly, mg per 100 ml	on wet digest, mg per 100 ml	Calcium found by oxalate titration,[1] mg per 100 ml	Specimen No.	directly, mg per 100 ml	on wet digest, mg per 100 ml	Calcium found by oxalate titration,[1] mg per 100 ml
1	8·1	8·0	8·1	26	8·7	9·1	9·0
2	8·9	9·4	8·8	27	10·0	9·5	9·4
3	9·4	9·4	9·4	28	9·3	8·7	9·1
4	10·3	10·2	10·2	29	8·7	8·4	8·8
5	10·3	9·8	10·4	30	9·0	8·7	8·6
6	8·4	8·2	8·2	31	9·5	9·4	9·8
7	10·2	10·1	10·3	32	8·9	9·2	8·9
8	8·9	9·2	9·1	33	9·4	9·9	9·9
9	9·8	9·9	9·9	34	9·0	9·4	9·1
10	10·4	10·1	9·8	35	9·3	9·6	10·1
11	9·4	9·7	9·1	36	9·0	9·2	9·1
12	10·1	9·7	9·3	37	8·8	9·1	9·0
13	9·8	9·8	9·6	38	9·8	9·8	9·5
14	9·8	10·4	10·2	39	9·8	8·8	9·5
15	10·2	10·7	10·3	40	7·9	8·2	8·1
16	8·4	8·6	8·5	41	8·7	8·5	8·8
17	8·6	8·2	8·4	42	9·9	10·1	10·0
18	9·8	9·6	9·9	43	8·6	8·8	8·6
19	9·8	10·1	10·0	44	10·0	9·8	10·0
20	9·7	9·5	9·5	45	9·4	9·3	9·4
21	8·8	8·7	9·0	46	10·0	10·0	9·9
22	8·4	9·2	8·8	47	9·8	9·5	9·5
23	9·1	9·6	9·0	48	10·4	10·2	10·3
24	8·5	8·4	8·3	49	9·6	9·6	9·7
25	14·1	14·0	13·7	50	10·3	10·0	10·3
			Mean for all specimens ..		9·46	9·47	9·46

The method was compared with Clark and Collip's oxalate-titration method.[1] Simultaneously, 0·2-ml portions of serum were digested with 0·5 ml of concentrated nitric acid and 0·1 ml of 60 per cent. w/w perchloric acid; after being heated until all the nitric acid had been removed and the final solutions were clear and colourless, 0·15 ml of ethanolamine and 1 ml

of a 1 per cent. w/v solution of tartaric acid were added to each sample digest and the resultant solutions were analysed for calcium by the proposed method. The results are shown in Table II and indicate close agreement between the methods.

The possible variation caused by drainage was also studied. A 5-ml portion of the calcium reagent solution contains sufficient naphthalhydroxamic acid to precipitate 120 μg of calcium. The amounts of liquid remaining after drainage under the conditions of the determination are shown in Table III. The range of variation was 0·012 to 0·029 ml for blank, standard or test solutions, and the maximum error caused by drainage variations, therefore, was $\pm$0·2 μg of calcium, equivalent to an error in serum calcium of $\pm$0·1 mg per 100 ml when 0·2-ml portions are used in the determination.

TABLE III

VOLUME REMAINING IN CYLINDRICAL 15-ml CENTRIFUGE TUBES AFTER DRAINAGE

Each tube originally contained 5·2 ml of liquid and was allowed to drain for 5 minutes under test conditions

Liquid present	Number of tests	Residual volume in tube	
		Mean, ml	Range, ml
Water	22	0·0161	0·012 to 0·021
Calcium standard, 10 mg per 100 ml ..	24	0·0190	0·013 to 0·026
Calcium standard, 40 mg per 100 ml ..	11	0·0230	0·021 to 0·029
Serum	50	0·0247	0·021 to 0·029

DISCUSSION OF THE METHOD

Several workers have recently criticised the standard oxalate method for determining calcium in serum and have stated that the different results obtained by using their own methods were the correct ones. Wilkinson[8] titrated 0·1 ml of serum directly with EDTA solution, with murexide as indicator and photometric observation of the end-point; his mean results were 0·7 to 0·8 mg of calcium per 100 ml lower than those found by a modification[9] of Clark and Collip's oxalate method. MacIntyre,[5] by high-temperature flame spectrophotometry of protein-free extracts, obtained results averaging 0·4 mg of calcium per 100 ml higher than those given by a modification of Tisdall's[10] oxalate method recommended by King.[11] MacIntyre's paper includes a study of this particular oxalate method; by using radioactive tracers, he found that 1·3 per cent. of the calcium was not precipitated and that 5·5 per cent. was lost in washing the precipitate. Allowing for some co-precipitation of magnesium, MacIntyre concluded that the net average loss of calcium was 4 per cent. or 0·4 mg per 100 ml. On this evidence, he considered that his results were the correct ones. The reference method chosen by MacIntyre involves two washes of the precipitate, which is suspended during the process. Tisdall also used two washes, but did not disturb the precipitate during washing, and it is probable that most of the calcium that MacIntyre proved to be lost was dissolved during the second wash, when the protective action of the common oxalate ion would be negligible. Clark and Collip's method involves only one wash, and it seems likely that, had MacIntyre used this method for comparison, he would have obtained results in closer agreement with his flame-spectrophotometric method. In this connection, it is interesting to note that King and Wootton[12] recommend an oxalate-precipitation method that involves only one wash and is similar to Clark and Collip's method.[1]

Since the proposed procedure compares well with Clark and Collip's oxalate method and gives identical results whether carried out directly on serum or on a wet digest, it appears to be a reliable and convenient method for determining calcium in small amounts of serum. The method was developed for serum, but heparinised plasma gives identical results and urine may also be analysed. A 24-hour specimen collected with 10 ml of concentrated hydrochloric acid as a preservative should be diluted to 2 litres with distilled water, and 0·2 ml of urine (cleared by centrifugation) should be taken for analysis. The small amount of acid in the sample does not interfere with the determination, which should be repeated with larger or smaller amounts of urine if the final optical density is less than 0·2 or greater than 0·8. The level of magnesium in a 24-hour urine sample diluted to 2 litres may occasionally exceed 10 mg per 100 ml. The results will then be slightly increased; when the urine calcium is 10 mg per 100 ml, this increase is equivalent to about 0·2 mg of calcium per 100 ml for each

additional 10 mg of magnesium per 100 ml. Table IV shows a comparison between results for calcium in urine by the proposed method and by applying Clark and Collip's method to clear urine adjusted to a pH between 4 and 5, as advocated by Varley.[13]

TABLE IV

COMPARISON OF RESULTS FOR URINE CALCIUM

Determinations by Clark and Collip's method were made on urine adjusted to a pH between 4 and 5

Specimen No.	Calcium found by— proposed method, mg per 100 ml	Clark and Collip's method,[1] mg per 100 ml	Specimen No.	Calcium found by— proposed method, mg per 100 ml	Clark and Collip's method,[1] mg per 100 ml
1	21·8	20·6	11	10·0	9·8
2	10·6	11·6	12	12·8	11·4
3	1·2	1·3	13	5·8	5·5
4	7·3	6·9	14	15·9	14·3
5	2·1	2·1	15	23·0	23·0
6	15·8	16·4	16	10·2	9·4
7	15·9	15·4	17	12·9	12·1
8	8·0	8·1	18	50·7	49·6
9	1·2	1·3	19	32·8	34·0
10	18·6	19·4	20	7·3	7·6
		Mean for all specimens	..	14·20	13·99

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Determination of Terminal Epoxides, Particularly Styrene Oxide

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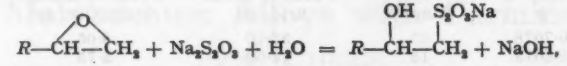
Compounds having terminal epoxy groups can be determined by titrating the alkali liberated on reaction with sodium thiosulphate in a solvent such as aqueous acetone. The alkali must be titrated continuously to prevent its neutralisation by further reaction, a procedure that is tedious, but, with appropriate precautions, can be fairly accurate. An equally accurate but simpler and more rapid method is described, in which the reaction takes place in the presence of magnesium sulphate and a solvent such as ethanol. The pH is thus prevented from rising by the precipitation of magnesium hydroxide, which, in the presence of sufficient ethanol, is so insoluble that the solution remains acid to phenolphthalein. Under these conditions the reaction is still quantitative, even when carried out for a prolonged period at the boiling-point of the solution. Water is added at the completion of the reaction so that the magnesium hydroxide becomes sufficiently soluble for titration with acetic acid, phenolphthalein being used as indicator.

Lauer and Hill[1] and Culvenor, Davies and Heath[2] studied the reaction of ethylene oxide with inorganic salts containing sulphur, including sodium sulphite and sodium thiosulphate, and Ross[3] showed that certain higher epoxides could be determined quantitatively by titrating the alkali liberated when the epoxide reacts with sodium thiosulphate. Swan[4] described the disadvantages of the thiosulphate method and proposed the use of sodium sulphite, which gives a similar reaction with the liberation of alkali, but has the disadvantage that aldehydes and some ketones interfere.

Some preliminary experiments were made to investigate the reaction conditions for the quantitative determination of styrene oxide. The desired reaction is—



and it is assumed that the sulphur is attached to the β-carbon. If the alkali is allowed to accumulate, the organic thiosulphate compound decomposes further with the liberation of acid, which neutralises the alkali liberated during the first stage. The second-stage reaction is avoided completely if the alkali from the first stage is neutralised as it is liberated. Experiment showed that the alkali can be neutralised sufficiently rapidly if the reaction is allowed to proceed over about 1 hour, which means that the temperature should not exceed about 40° C.

The method described in this paper avoids this limitation, the key step being the addition of magnesium sulphate to limit the rise in pH. This is particularly effective in the presence of a relatively high proportion of ethanol, because the solubility of magnesium hydroxide is lowered to such an extent that it does not dissolve in the hot solvent sufficiently to give a pink colour with phenolphthalein. On cooling, a faint pink colour develops, which becomes more intense when water is added. In this way, the reaction takes place under less alkaline conditions, and there is no loss of the liberated alkali even at the boiling-point of the solvent. A disadvantage is that the magnesium compound is precipitated on the side of the flask in a gelatinous form that does not dissolve rapidly in a solution only just acid to phenolphthalein, so that the direct titration is slow. Therefore, an excess of standard acetic acid is added and the excess is titrated when all the precipitate has dissolved. Care is necessary, because the precipitate adhering to the glass is almost transparent and may not be noticed unless the flask is tilted.

Method

Reagents—

Magnesium sulphate - sodium thiosulphate solution—Dissolve 12·5 g of magnesium sulphate crystals and 12·5 g of sodium thiosulphate crystals in water, and dilute to 100 ml.

Acetic acid, 0·2 N.

Sodium hydroxide, 0·1 N.

Screened phenolphthalein - methyl red indicator solution—Prepare a solution of 1 g of phenolphthalein, 0·02 g of methyl red and 0·01 g of methylene blue in 100 ml of ethanol. The indicator has end-points at pH 5·4 and 8·25.

PROCEDURE—

In a conical flask place 40 ml of magnesium sulphate - sodium thiosulphate solution, 20 ml of ethanol and 1 ml of screened phenolphthalein - methyl red indicator solution. Adjust to pH 8·25 with 0·1 *N* sodium hydroxide, and add the weighed sample containing 0·5 to 0·7 g of styrene oxide. Boil under reflux for 15 minutes, cool, add 30 ml of distilled water, and titrate with 0·2 *N* acetic acid. To ensure complete solution of the precipitated magnesium hydroxide, particularly that adhering to the glass, add an excess of about 5 ml of 0·2 *N* acetic acid, a proportion that, in the presence of sodium acetate, will not be sufficient to lower the pH enough to cause decomposition of the sodium thiosulphate. The second end-point of the indicator at pH 5·4 is an additional precaution. When the precipitate has completely dissolved, titrate the excess of acetic acid with 0·1 *N* sodium hydroxide. The accuracy may be increased by carrying out a blank determination, for which a volume of water equal to the epoxide titre should be added, so that the end-point is measured in the same concentration of ethanol.

RESULTS

The results in Table I were obtained with a specimen of styrene oxide that had been purified by vacuum distillation after recrystallisation from light petroleum, cooling having been carried out with a mixture of solid carbon dioxide and acetone. No appreciable impurities were detected by gas - liquid chromatography.

TABLE I

DETERMINATION OF STYRENE OXIDE

Experiment No.	Weight of sample, g	Reaction time, minutes	Titre of 0·2020 *N* acetic acid, ml	Titre of 0·0990 *N* sodium hydroxide, ml	Styrene oxide content, %
1	0·7078	15	32·10	6·05	99·90
2	0·4073	15	18·50	3·75	99·28
3	0·2927	15	13·80	3·70	99·38
4	0·4467	15	20·50	4·30	99·92
5	0·3816	15	17·90	4·50	99·81
6	0·5692	120	24·00	1·55	99·09
7	0·4702	120	20·95	3·80	98·52
8	0·6347	5	29·40	7·05	99·20
9	0·4295	5	21·10	7·15	99·42

Experiments 1 to 5 were made under standard conditions with a reaction time of 15 minutes. The efficiency with which the undesired side reaction has been eliminated is shown by the results of experiments 6 to 9, in which the reaction time varied from 5 minutes to 2 hours. Under the same conditions with a reaction time of 15 minutes, but in the absence of magnesium sulphate, the corresponding values in two titrations were 25·8 and 15·8 per cent. of styrene oxide.

The method has been used with other terminal oxides, but styrene oxide was the only one of the higher epoxides in a pure state. For example, a sample of 2-hydroxy-2:6-dimethyl-7:8-epoxyoctane had an estimated purity of 94 per cent., a value not inconsistent with that found by gas - liquid chromatography. Some caution is needed when dealing with unknown epoxides, because the reaction is sometimes slow and does not appear to be specific for the terminal group. For example, a mixture of 1:2- and 2:3-butylene oxide gave a titre that exceeded the estimated 1:2-butylene oxide content. On the other hand, commercial samples of epoxidised soya-bean oil gave no titre, even after boiling for 24 hours. The reaction times may vary widely; it was found that a commercial sample of 1:2-octylene oxide reacted very slowly. After boiling the solution for 1 hour, the alkali liberated corresponded to 40 per cent. of octylene oxide; after boiling overnight, the alkali was equivalent to 80 per cent., but this result was still thought to be too low.

A commercial sample of epoxy-α-olefin made by the epoxidation of a mixture of straight-chain olefins containing 16 to 18 carbon atoms and having terminal double bonds gave the results shown below—

Reflux time, hours	¼	1	16¼	23	48
Epoxide (as $C_{16\cdot5}H_{33}O$), %	3·2	7·4	67·4	77·5	73·5

Comparison of the results after 23 and 48 hours shows that the maximum titre must be corrected for the alkali consumed in side reactions. The total epoxide content of the same sample determined on the same basis by the pyridine - hydrochloric acid method was 83·6 per cent.[5]

A commercial sample of epoxy-olefin made from the same olefin mixture as the previous example, but in which the olefin was isomerised before epoxidation, gave a titre corresponding to 2·1 per cent. of terminal epoxide after 24 hours by the thiosulphate method, and a total epoxide content of 85·2 per cent. by the pyridine - hydrochloric acid method.

The thiosulphate method described has been used mainly for quality control during the manufacture of styrene oxide.

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The Spectrophotometric Determination of Boron in Molybdenum Alloys with Carmine

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The conditions controlling the development of colour between boric acid and carmine red have been studied, with particular reference to the concentrations of sulphuric acid, chloride, molybdenum, reagent and alloying elements. A method is outlined for the direct determination of boron in molybdenum alloys, over the range 0·01 to 0·20 per cent., measurements being made spectrophotometrically at 610 mμ. The mean error over this range was found to be ±0·006 per cent. of boron.

Early in the metallurgical investigations of the physical properties of pure molybdenum and of its alloys with titanium, zirconium, etc., the need arose for a good de-oxidising agent. Boron was one of the elements used to fulfil this function. It was expected that the residual boron content might fall within the range 0·01 to 0·20 per cent., more particularly so since the mechanical losses peculiar to the method of fabrication were unknown. The amount of sample available for analysis was small, usually not more than a few grams, hence a colorimetric method was the obvious choice for determination of the boron.

The colorimetric determination of boron has been conducted with several reagents, the most popular of which appear to be quinalizarin,[1,2,3] curcumin,[4] carmine[5] and 1 : 1′-dianthrimide.[6,7] Experiments were carried out to determine their suitability for the direct determination of boron in the presence of molybdenum.

Quinalizarin in concentrated sulphuric acid was found to give a peak absorption for the reagent at 570 mμ; that for the boron complex was at 590 mμ. The colour was found to obey Beer's law only over a limited range of concentrations, and the investigation was not pursued.

Silverman and Trego's method,[4] based on a 40/60 aqueous acetone solution of curcumin, yielded solutions that obeyed Beer's law over a wide range of concentrations, but, when these preliminary experiments were repeated in the presence of 10 mg of molybdenum, no colour was obtained. Separation of the molybdenum by precipitation from thiomolybdate solution permitted the re-development of the boron colour, but, as a direct method of determination was required, no further work was carried out with this reagent.

The reagent dianthrimide requires that the sample solution be treated with calcium hydroxide, and then evaporated to dryness and re-dissolved in concentrated sulphuric acid for final colour development. It was considered that the need for 100 per cent. v/v sulphuric acid, coupled with its strong tendency to form blue reduction products with solid molybdates and the recommended narrow range of 0 to 10 μg of boron, did not justify the use of this reagent, despite its greater sensitivity.

Hatcher and Wilcox[5] proposed the use of carminic acid (carmine red) in 91 per cent. v/v sulphuric acid for determining boron in soils and waters. These workers stated that under the conditions of their determination no interference was caused by small amounts of molybdenum. This reagent was therefore chosen for further study.

After completion of the work described in this paper, which was carried out during 1956 to 1958, carmine was recommended by Hopkin and Williams Ltd.,[8] who put forward the conditions devised by Hatcher and Wilcox,[5] and also by Callicoat and Wolszon.[9] The latter workers investigated some of the variables covered in this paper, with substantially the same result, in addition to which they observed variations in reaction sensitivity between different grades of reagent. It should be pointed out that the findings of the various workers differ quantitatively with respect to sensitivity and time of colour formation, probably as a result of using reagents of different qualities. Unless an identical supply of a particular grade of carmine is used, it is possible that independent workers will not corroborate one another's findings in every respect. Throughout the work described here the ordinary reagent grade of carmine, obtained from Hopkin and Williams Ltd., was used. Periodic checks with 10 μg of boron under standard conditions provided no evidence that the reagent solution of carmine in sulphuric acid had changed over a period 1 to 2 months, during which most of this work was completed. In later work, a reagent solution about 18 months old was found to have lost 11 per cent. of its original sensitivity, as assessed by the optical density obtained with a fixed weight of boron.

Experimental

Wavelength for maximum sensitivity—

Solutions were prepared simulating Hatcher and Wilcox's conditions (91 per cent. v/v sulphuric acid and 0·02 per cent. w/v carmine reagent solution) and readings were taken with

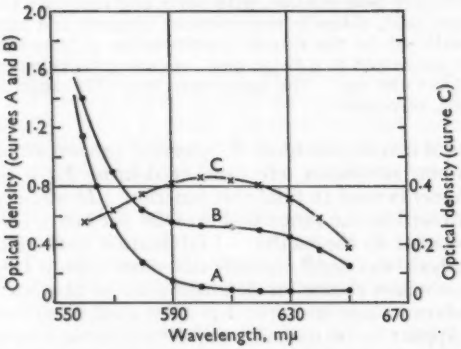

Fig. 1. Absorption curves of carmine reagent and the boron - carmine compound: curve A, no boron; curve B, 0·8 μg of boron per ml; curve C, difference plot.

Solutions contained 0·02 per cent. w/v of carmine in 91 per cent. v/v sulphuric acid and optical densities for curves A and B were measured after 100 minutes' standing time in 1-cm cells against water; the extinction coefficient at 610 mμ was 537

a Unicam SP500 spectrophotometer after colour development for 100 minutes, since preliminary observations had indicated that the period of 45 minutes recommended by Hatcher and Wilcox was not long enough to yield maximum colour formation. A graph showing the relationship between optical density and wavelength is shown in Fig. 1, and it is evident from the differential curve that the peak for $E_{max.}$ is very broad. In fact, the evidence from many similar differential curves would suggest that any wavelength between 590 and 620 mμ would be equally satisfactory; throughout the remainder of the work reported in this paper 610 mμ has been employed.

RATE OF COLOUR DEVELOPMENT—

The optical densities of solutions, prepared in a similar manner, were measured at frequent intervals over a period of 3 hours at 610 mμ. One solution not containing boron gave a constant reading over the whole period, whereas the remaining solutions, all containing boron, had an extremely slow rate of colour development. Ideally, a standing period of many hours is to be preferred for full colour development, but practical considerations require a shorter time; a period of 3 hours was adopted for subsequent investigations.

ACIDITY—

A series of solutions was prepared by adding a fixed amount (25 μg) of boron, as sodium metaborate, to different, but accurately measured, amounts of water. To each of these solutions were added 10 ml of sulphuric acid, sp.gr. 1·84, and 10 ml of a carmine solution (0·05 per cent. w/v in concentrated sulphuric acid), and the volumes were adjusted to 25 ml with concentrated sulphuric acid. The time was noted and the solutions were cooled in a water bath. When the solutions were cold the volumes were again adjusted to 25 ml with sulphuric acid to compensate for the contraction on cooling, and the solutions were then mixed thoroughly. The optical densities were measured at intervals of 10 minutes over a period of 3 hours. The values obtained are shown graphically in Fig. 2, from which it can be seen that maximum sensitivity is obtained at 92 per cent. v/v sulphuric acid (sp.gr. 1·84).

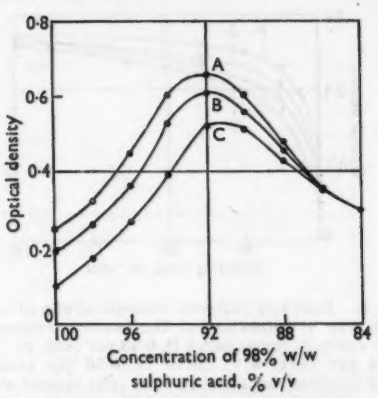

Fig. 2. Effect of acidity on formation of the boron - carmine compound. Optical densities measured in 1-cm cells against water at 610 mμ after different standing times: curve A, 180 minutes; curve B, 100 minutes; curve C, 40 minutes. Solutions contained 0·02 per cent. w/v of carmine and 1·0 μg of boron per ml

DYE CONCENTRATION—

Experiments showed that the rate of colour development was influenced by the dye concentration, the rate of colour formation being extremely slow at low dye concentrations. The maximum colour that could be obtained after an extremely long period of standing was approximately proportional to the dye concentration. Thus high sensitivity and rapid colour formation are associated with high dye concentration; against this, however, must be set the loss in precision owing to the high optical density of the blank solution.

The choice of the reagent strength is therefore at all times a compromise between the conflicting advantages and disadvantages, and the concentration employed through this work (0·02 per cent. w/v) is considered to be the most suitable.

TEMPERATURE CONTROL—

The experimental conditions result in evolution of heat during the early stages of colour development and strict temperature control over the first 10 to 15 minutes is thus impossible. However, provided that the same procedure is followed in all experiments, the results should be reproducible. No major effort was made to study the effect of temperature on the rate of colour formation, but some evidence that temperature control is not critical is afforded by the over-all precision of results, set out in later sections, when standard conditions were employed.

CHLORIDE CONCENTRATION—

Preliminary experiments showed that the rate of colour development was retarded by the presence of chloride ions and, since hydrochloric acid was essential for the initial solution of the molybdenum samples, the effect of this ion was determined at several concentration levels.

Determination of the chloride ion concentration in solutions prepared as described under "Procedure" showed that the final chloride concentration was of the order of 0·40 per cent. w/v. A series of solutions to cover a range of chloride concentrations up to 0·86 per cent. w/v was prepared by adding constant-boiling hydrochloric acid to several solutions, each containing 20 μg of boron. After colour development, readings were taken at 610 mμ at intervals of 10 minutes over a period of 3 hours. It is clear from the results obtained, shown graphically in Fig. 3, that an increase in chloride ion concentration results in a progressive decrease in the rate of colour formation. After a period of 90 minutes, the optical density of a solution containing 0·86 per cent. w/v of chloride differs by 10 per cent. from that of the solution containing none. It is important therefore that the time of standing and the chloride ion concentration should be similar for both calibration and test solutions.

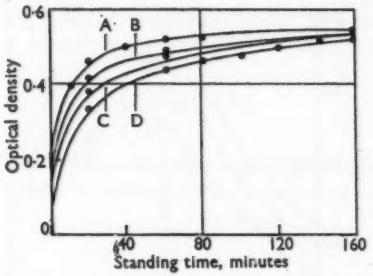

Fig. 3. Effect of different concentrations of chloride on the rate of formation of the boron - carmine compound: curve A, none; curve B, 0·43 per cent. w/v; curve C, 0·64 per cent. w/v; curve D, 0·86 per cent. w/v. Optical densities measured in 1-cm cells against water at 610 mμ; solutions contained 0·02 per cent. w/v of carmine in 92 per cent. v/v sulphuric acid and 0·8 μg of boron per ml

MOLYBDENUM—

With boron-free molybdenum a series of solutions was prepared containing 0, 5, 10 and 15 mg of molybdenum and 10 μg of boron in the 2-ml aliquot used for colour development. The chloride ion concentration was adjusted to be equivalent to a final concentration of 0·40 per cent. w/v. The colour was developed as described under "Procedure," and the optical density was measured at 610 mμ at 10-minute intervals for a period of 3 hours. It can be seen from the graphical expression of the results in Fig. 4 that the presence of molybdenum suppresses the colour obtained from the boron. A plot of the relative sensitivities obtained for the 90-minute period is given in Fig. 5, from which it may be calculated that a $\pm$50 per cent. change in the molybdenum concentration at the 0·4 mg per ml level results in an average

error of 20 per cent. in the boron determination. It is clear that no serious error is involved by a 10 per cent. decrease in the molybdenum content due to the presence of an alloying constituent, provided that the alloying element itself does not interfere with the boron - carmine colour.

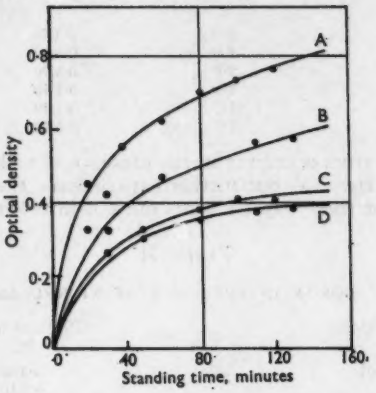

Fig. 4. Effect of molybdenum on the rate of formation of the boron - carmine compound: curve A, none; curve B, 0·2 mg per ml; curve C, 0·4 mg per ml; curve D, 0·6 mg per ml. Optical densities measured in 4-cm cells against the reagent blank solution at 610 mμ; solutions contained 0·02 per cent. w/v of carmine in 92 per cent. v/v sulphuric acid, 0·4 per cent. w/v of chloride and 0·4 μg of boron per ml

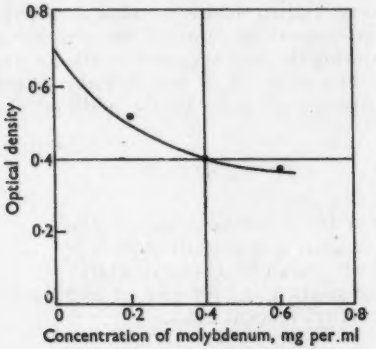

Fig. 5. Effect of molybdenum on sensitivity after standing for 90 minutes. Optical densities measured in 4-cm cells against the reagent blank solution at 610 mμ; solutions contained 0·02 per cent. w/v of carmine in 92 per cent. v/v sulphuric acid, 0·4 per cent. w/v of chloride and 0·4 μg of boron per ml

ALLOYING ELEMENTS—

To determine the effect of alloying elements, single synthetic solutions were prepared containing the chlorides of copper, chromium, iron, nickel, titanium or zirconium, equivalent to an alloying content of about 2 per cent. The recoveries of boron, determined in the presence of these elements, are shown in Table I, and it can be seen that no serious error is attributable to these alloying additions, at the concentration levels considered.

TABLE I

RECOVERY OF ADDED BORON IN PRESENCE OF ALLOYING ELEMENTS

Metal added	Amount of metal added, %	Boron added, %	Boron found, %
Copper	2·0	0·150	0·154
Chromium	2·0	0·150	0·160
Iron	2·0	0·150	0·156
Nickel	2·0	0·150	0·159
Titanium	1·5	0·150	0·147
Zirconium	1·5	0·150	0·145

The magnitude of the errors occurring in the presence of nickel and chromium suggested the possibility of slight interference, but further experiments, the results of which are shown in Table II, established that these two elements did not interfere, even when present at the 10 per cent. level.

TABLE II

RECOVERY OF BORON IN PRESENCE OF NICKEL AND CHROMIUM

Conditions	Boron added, %	Boron found, %
0·010 g of molybdenum per 25 ml	0·150	0·149, 0·152, 0·150
	0·010	0·010, 0·010, 0·011
0·010 g of molybdenum and 0·0010 g of nickel per 25 ml ..	0·150	0·140, 0·153, 0·150
	0·010	0·012, 0·012, 0·018
0·010 g of molybdenum and 0·0010 g of chromium (as Cr^{3+}) per 25 ml	0·150	0·150, 0·153, 0·157
	0·010	0·013, 0·017, 0·013

NITRIC ACID—

It was found that excessive amounts of nitric acid and the presence of nitrous acid were responsible for the bleaching of the carmine colour noticed in earlier experiments. To ensure that the nitric acid was kept at a minimum, only sufficient acid was added to oxidise the sample, and this concentration of nitric acid was reduced subsequently by a factor of 20 when taking an aliquot for the determination. Most of the nitrogen peroxide is expelled from the top of the reflux condenser during the final stages of solution of the sample, but, since complete expulsion might also involve loss of boron, it was desirable to use other means to destroy the remaining nitrous acid. This was achieved by the addition of 0·5 g of urea to the solution before dilution.

METHOD

REAGENTS—

Hydrochloric acid, sp.gr. 1·18—Analytical-reagent grade.
Nitric acid, sp.gr. 1·42—Analytical-reagent grade.
Sulphuric acid, sp.gr. 1·84—Analytical-reagent grade.
Phenolphthalein indicator solution, 0·1 per cent. in 50 per cent. ethanol.
Sodium carbonate—Analytical-reagent grade.
Urea—Tabloid 0·5 g.
Carmine reagent solution, 0·05 per cent. w/v in sulphuric acid, sp.gr. 1·84.
Standard boron solution A—Dissolve 0·2206 g of analytical-reagent grade sodium borate decahydrate in distilled water, and dilute to the mark in a 250-ml calibrated flask.

$$1 \text{ ml} \equiv 1{\cdot}0 \times 10^{-4} \text{ g of boron.}$$

Standard boron solution B—Dilute a 20-ml portion of solution A to the mark in a 1000-ml calibrated flask.

$$1 \text{ ml} \equiv 2{\cdot}0 \times 10^{-6} \text{ g of boron.}$$

APPARATUS—

(*a*) All-glass boron-free 100-ml flask and water-cooled reflux condenser fitted with a standard B19 cone and socket.

(*b*) Unicam SP500 spectrophotometer or its equivalent.

PROCEDURE—

Weigh out 0·200 g of sample, and transfer to a 100-ml flask fitted with a B19 socket; connect the flask to a water-cooled reflux condenser. Turn on the water supply, and add through the condenser 10 ml of distilled water, 5 ml of hydrochloric acid and 12 to 15 drops of nitric acid; heat gently to assist solution. When solution is complete the liquid should be free from any brown coloration. If a brown colour persists it should be discharged by boiling with further dropwise additions of nitric acid. Cool the flask, wash down the condenser with distilled water, disconnect the flask, add 0·5 g of urea, dilute to 100 ml in a calibrated flask, and mix thoroughly.

Transfer a 5-ml aliquot of solution to a 50-ml porcelain crucible. Add 1 drop of phenolphthalein indicator solution and then sufficient sodium carbonate to give the solution a permanent pink colour. Place the crucible over a conical beaker containing boiling water, and evaporate to dryness. Cool, re-dissolve the salts in 2·0 ml of distilled water, add 10 ml of concentrated sulphuric acid, swirling the contents of the crucible to avoid local boiling, and again cool. Place a small funnel in the mouth of a clean dry 25-ml calibrated flask, and pour the contents of the crucible into the funnel. Fill a 10-ml pipette with carmine reagent solution, and direct the contents of the pipette in such a manner that the whole of the crucible is flushed out, to ensure complete removal of the sample; allow the pipette to drain in the usual manner, and finally wash the crucible with a few millilitres of sulphuric acid. Cool the contents of the flask to room temperature with the minimum of delay, and then adjust the volume to the graduation mark with sulphuric acid. Mix thoroughly, and set aside for 90 minutes to allow the colour to develop. Meanwhile, prepare the requisite number of clean dry 40-mm absorption cells. About 5 minutes before the end of the colour-development period, transfer the solutions to the prepared cells, place in the cell holder, and allow any bubbles formed to rise to the surface; should any bubbles adhere to the optical faces, remove by giving the cell holder several sharp taps. Measure the optical density at 610 mμ against a blank solution prepared by taking 0·2 g of boron-free molybdenum through all stages of the procedure.

PREPARATION OF CALIBRATION GRAPH—

Dissolve 0·200 g of boron-free molybdenum as described under "Procedure," and dilute to 100 ml. Transfer 5-ml aliquots to each of seven 50-ml crucibles, and add to each 0, 1, 2, 5, 8, 10 and 12 ml of standard boron solution B. Add 1 drop of phenolphthalein indicator, and continue the procedure. Measure the optical density in 40-mm cells at 610 mμ against the blank solution. The graphical expression of results obtained from a typical calibration series gave the figures below—

Number of results	6
Mean boron added, %	0·1267
Mean boron recovered, %	0·1275
Standard deviation, %	0·0056
Coefficient of variation, %	4·3

ACCURACY OF THE METHOD

ADDITIONS OF BORON TO MOLYBDENUM METAL—

To assess the accuracy of the method, known amounts of a molybdenum alloy containing 0·34 per cent. of boron (determined by Bush and Higg's method[10]) were dissolved together with pure molybdenum metal and submitted to the proposed procedure. The values obtained by reference to the calibration graph are shown in Table III.

TABLE III

RESULTS ON MIXTURES OF MOLYBDENUM AND MOLYBDENUM - BORON ALLOYS

Pure molybdenum taken, g	Molybdenum - boron alloy taken, g	Boron present, %	Boron found, %	Difference, %	Standard deviation, %
0·0784	0·1216	0·207	0·203	−0·004	
0·0981	0·1019	0·173	0·167	−0·006	
0·1265	0·0735	0·125	0·130	+0·005	±0·0075
0·1392	0·0608	0·103	0·116	+0·013	
0·1756	0·0244	0·042	0·047	+0·005	
0·1854	0·0156	0·027	0·025	−0·002	

The standard deviation of the recovery values in column 5 is ±0·0075 per cent. of boron, which agrees fairly well with that obtained during calibration. Known additions of boron were made to many alloys submitted for routine analysis, and the recovery values were obtained by difference. A representative selection of results is shown in Table IV, from which it can be seen that the mean deviation is again in agreement with earlier values.

TABLE IV

RESULTS ON MIXTURES OF MOLYBDENUM ALLOYS AND BORIC ACID

Boron added, %	Boron found, %	Difference, %	Mean, %	Standard deviation, %
0·020	0·022	+0·002		
0·040	0·041	+0·001		
0·100	0·113	+0·013		
0·160	0·154	−0·006		
0·240	0·237	−0·003		
0·280	0·278	−0·002		
0·400	0·406	+0·006		
0·100	0·093	−0·007		
0·060	0·057	−0·003	±0·006	±0·0073
0·140	0·126	−0·014		
0·200	0·200	Nil		
0·040	0·041	+0·001		
0·080	0·092	+0·012		
0·160	0·170	+0·010		
0·260	0·266	+0·006		
0·150	0·160	+0·010		

CONCLUSION

The investigation has shown that the colorimetric determination of boron with a solution of carmine red in sulphuric acid may be applied directly to the analysis of molybdenum and its alloys without prior separation of the molybdenum. Over the range 0·01 to 0·20 per cent. of boron, the mean error was found to be ±0·006 per cent. of boron.

I thank Mr. G. H. Bush and Mr. A. Bacon for their keen interest, constructive criticisms and help generously extended during the preparation of this report, which is reproduced by permission of the Controller of H.M. Stationery Office.

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The Effect of Substituent Groupings on the Chromatographic Behaviour of Phenoxyacetic Acids

Part II.* Chloro-substituted Phenoxyacetic Acids

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and R. J. T. GRAHAM

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Twelve chloro-substituted phenoxyacetic acids were chromatographed under standardised conditions. From a comparison of the results obtained for chloro-, methyl- and some methoxy-substituted acids, it is suggested that the acid may be adsorbed on to the paper by attachment of both the acid group and the substituent group to the cellulose, the substituent group being attached by hydrogen bonding between the hydroxyl groups of the cellulose and the electronegative atom of the substituent. The relatively low adsorption of acids substituted in both 2- and 6-positions is probably caused by restriction of rotation of the phenoxyacetic acid grouping, resulting in attachment by this group only.

In an earlier paper[1] describing the behaviour of methyl-substituted phenoxyacetic acids chromatographed under rigorously standardised conditions, we suggested that the R_F value and hence the R_M value of an acid was due to four factors—

(*i*) The behaviour of the fundamental group—the acid grouping.
(*ii*) The number of substituent groups.
(*iii*) The relative positions of the fundamental group and the substituent group or groups.
(*iv*) The relative positions of the substituent groups.

Martin[2] predicted that the addition of a group to a molecule should have an effect upon the adsorption of that molecule, independent of the rest of the molecule. In the methyl-substituted acids this was not so; the results obtained showed that for isomers the positions of the substituent groups had noticeable effects. The greater the distance of the methyl group from the phenoxyacetic acid grouping, the smaller was the change in the R_M value of the acid, when compared with phenoxyacetic acid. The nearer the substituent groups were to one another, the smaller was the change in R_M. These effects were regarded as complementary, the major effect being the relative positions of the substituent group and the fundamental group.

No explanation of these effects was then attempted; the apparently anomalous behaviour of the acids substituted in both 2- and 6-positions was suggested to be caused by some steric inhibition of the activity of the fundamental group.

It was considered that the use of a substituent group larger than the methyl group should enhance any steric effects and possibly permit an explanation of the effects to be given.

Thus a series of chloro-substituted phenoxyacetic acids was prepared and chromatographed under conditions identical to those used for the methyl acids. The *n*-butyl alcohol, the basis of the solvent systems used, was shown by vapour-phase chromatography to be identical in both series.

The results are shown in Table I. Each value is the mean of three or four results obtained from papers on which the R_F value of the standard phenoxyacetic acid used did not vary by more than 0·02 unit of R_F from a pre-determined mean.[1] All the acids used were chromatographically pure, only one spot of fairly uniform shape being obtained on development.

* For details of Part I of this series, see reference list, p. 907.

TABLE I

VALUES OF R_F AND R_M FOR CHLORO-SUBSTITUTED ACIDS IN SYSTEMS CONTAINING *n*-BUTYL ALCOHOL

Acid	Solvent A*		Solvent B†	
	R_F	R_M	R_F	R_M
Phenoxyacetic	0·200	0·600	0·300	0·360
2-Chloro-	0·290	0·390	0·390	0·190
3-Chloro-	0·305	0·360	0·440	0·095
4-Chloro-	0·275	0·425	0·390	0·190
2:3-Dichloro-	0·375	0·220	0·455	0·080
2:4-Dichloro-	0·380	0·220	0·495	0·010
2:5-Dichloro-	0·400	0·175	0·525	−0·045
2:6-Dichloro-	0·425	0·130	0·525	−0·045
3:4-Dichloro-	0·410	0·160	0·500	0·000
3:5-Dichloro-	0·445	0·095	0·550	−0·100
2:4:5-Trichloro-	0·480	0·040	0·565	−0·115
2:4:6-Trichloro-	0·505	0·000	0·580	−0·155
Average mono-	0·290	0·390	0·405	0·170
Average di-	0·410	0·165	0·510	−0·015
Average tri-	0·490	0·015	0·575	−0·130

* *n*-Butyl alcohol saturated with water.
† *n*-Butyl alcohol saturated with 1·5 *N* ammonium hydroxide.

From the results obtained it can be seen that, to a first approximation only, does a substituent group have a purely additive effect on the R_F or the R_M value of the acids.

Comparison of the order of R_F values of the chloro- and methyl-substituted phenoxyacetic acids—

Methyl.....Unsubstituted <4<3<2<3:4<3:5<2:3<2:4<2:5<2:6<2:4:5<2:4:6

Chloro.....Unsubstituted <4<2<3<2:3<2:4<2:5≤3:4<2:6<3:5<2:4:5<2:4:6

suggests that substitution of a chlorine atom in a position *meta* to the phenoxyacetic acid grouping has a special effect on the mode of adsorption of the acid on to the paper.

We have supposed that it should be possible for the molecule to be attached to the paper, or the water therein, by the acid grouping and also by the substituent. From a consideration of three-dimensional models it appears that an acid could be attached by both the phenoxyacetic acid group and by the chloro group in all the mono-substituted acids.

For the 4-chloro-acid, the molecule could form an arch, being attached to the paper or the water by opposite ends of the molecule and would thus lie close to the cellulose molecules. For acids substituted in both 2- and 6-positions the substituent groups prevent rotation of the phenoxyacetic acid grouping, which is thus held fairly rigid and could cause the non-polar aromatic part of the molecule to be held away from the cellulose and in the non-polar solvent when the acid is adsorbed on to the paper through its carboxy group.

The low adsorption of the *meta*-substituted acids cannot be accounted for by any such steric effects, and it was thought that the reason was possibly the inductive effect of the chlorine atom affecting the strength of the acids and hence the ease of attachment by the carboxyl group. To obtain further evidence of the behaviour of acids substituted with relatively large substituent groups containing electronegative atoms, we prepared and chromatographed monomethoxyphenoxyacetic acids.

A comparison of the results obtained for these acids, the corresponding chloro- and methyl-acids and the dissociation constants of the nine acids is made in Table II.

TABLE II

COMPARISON OF RESULTS FOR DIFFERENT ACIDS

Position substituted	R_F values of acids: Solvent A			Solvent B			Dissociation constant[3] ($K \times 10^4$)		
	CH_3–	CH_3O–	Cl–	CH_3–	CH_3O–	Cl–	CH_3–	CH_3O–	Cl–
2–	0·330	0·170	0·290	0·395	0·215	0·390	5·93	5·88	8·90
3–	0·260	0·170	0·305	0·380	0·225	0·440	6·27	7·22	8·51
4–	0·245	0·135	0·275	0·380	0·165	0·390	6·09	6·13	7·89

From the results it appears that the degree of ionisation of the acid does not have a great effect on the adsorption of the acids, and that the low adsorption (R_F) of the 3- and the 3:5-substituted acids is not due to any effect on the acid strength.

We suggest that an explanation of the order of adsorption of the acids is that hydrogen bonding may occur between the electronegative atoms of the substituent and the cellulose or the water therein. The main bonding is however that between the phenoxyacetic acid grouping and the cellulose.

The nearer the main points of attachment are to one another then the greater is the adsorption. Thus the relative positions of the substituent groups is a minor factor, but the nearer they are to one another the greater is the probability of hydrogen bonding. The apparently anomalous behaviour of acids substituted in the 4- or the 2:6-positions is accounted for by the stereochemistry of the molecules.

The nature of the substituent group will obviously have some effect, since the amount of hydrogen bonding will be a function of the nature of the group. Investigations of the effect of the nature of the group are in progress.

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NOTE—Reference 1 is to Part I of this series.

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The Effect of Substituent Groupings on the Chromatographic Behaviour of Phenoxyacetic Acids

Part III.* The Effect of the Nature of the Substituent

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Para-substituted phenoxyacetic acids were chromatographed under standardised conditions. Results indicate that the greater the probability of hydrogen bonding between the substituent and the stationary phase the greater is the adsorption of the acid and hence the lower is the R_F value.

WE have earlier suggested[1] that an important factor in determining the adsorption of phenoxyacetic acids on to cellulose was the ability of the acid to attach itself to the cellulose by the carboxyl group and also the substituent group, the latter being bonded to the cellulose or to the water contained therein by hydrogen bonding.

In the 2:6-di-substituted acids considered,[1,2] steric effects play an important part in determining the adsorption; the phenoxyacetic acid grouping is held rigidly away from the aromatic nucleus and hence the acid may be attached to the paper only by the carboxylic acid group. In the monomethyl-, monomethoxyl- and monochloro-substituted phenoxyacetic acids chromatographed, the order of the R_F values of particular isomers varies, but the R_F of the acids substituted in the 4-position was always the smallest, indicating that the adsorption of these acids on to the cellulose was the greatest. This was taken to indicate that these molecules were probably attached to the cellulose by opposite ends of the molecule.

To investigate the effects of different substituents on the adsorption of the acids a series of mono-substituted phenoxyacetic acids was prepared and chromatographed under conditions standardised as in previous investigations.

* For details of Parts I and II of this series, see reference list, p. 908.

The substituents were chosen so that both effect of size and electronegativity of the substituent group could be considered. The results are shown in Table I. Each value is the mean of 3 or 4 results obtained from papers on which the R_F of a standard (phenoxyacetic acid) did not vary by more than 0·02 unit of R_F from a previously determined mean.[1] All the acids used were chromatographically pure, only one spot of fairly uniform shape being obtained on development. All the acids except 4-hydroxy- and 4-carboxymethoxy- (–O–CH_2–COOH), were applied as a 1 per cent. w/v solution in *n*-butyl alcohol[2]; these two acids were not soluble to this extent and a saturated solution of the acids in *n*-butyl alcohol was used.

TABLE I

R_F AND R_M VALUES OF 4-SUBSTITUTED PHENOXYACETIC ACIDS

Acid	Solvent A*		Solvent B†	
	R_F	R_M	R_F	R_M
Phenoxyacetic acid	0·200	0·600	0·300	0·360
4-Fluoro-	0·210	0·570	0·325	0·320
4-Chloro-	0·275	0·425	0·390	0·190
4-Bromo-	0·265	0·440	0·385	0·205
4-Iodo-	0·255	0·450	0·360	0·245
4-Methyl-	0·245	0·475	0·380	0·215
4-Methoxy-	0·135	0·805	0·170	0·670
4-tertiary Butyl-	0·210	0·570	0·375	0·220
4-Carboxymethoxy-	0·050	1·280	No movement	
4-Hydroxy-	No movement		No movement	
4-Nitro-	0·165	0·705	0·230	0·525

* *n*-Butyl alcohol saturated with water.
† *n*-Butyl alcohol saturated with 1·5 *N* ammonium hydroxide.

The results indicate that the greater the electron density in the 4-position, the greater is the effect of the substituent.

The order of the effect of the halogens Cl>Br>I following the order of ease of polarisability of the halogen atoms is expected; the order F<Cl may be accounted for by the large inductive effect of the fluorine atom. Although the large mesomeric effect of the fluorine atom results in 4-fluorobenzoic acid having a lower dissociation constant than has 4-chlorobenzoic acid, the insulating effect of the ether oxygen of the phenoxyacetic acid grouping somewhat restricts the transference of the mesomeric effect to the carboxylic acid group, as shown by the dissociation constants of the acids[3]—

4-Fluorophenoxyacetic acid: $K = 7{\cdot}42 \times 10^{-4}$

4-Chlorophenoxyacetic acid: $K = 7{\cdot}89 \times 10^{-4}$

The order tertiary butyl>methyl is possibly due to the methyl group having a tendency to reduce its electron density by hyperconjugation with the benzene ring, whereas the tertiary butyl group has no such tendency.

Large movement of the hydroxy- and carboxymethoxy-acids is not to be expected, since they are probably attached by means of charged oxygen atoms; the nitro-group is possibly similarly attached.

The results indicate that the nature of the substituent group is of importance in determining the chromatographic behaviour of the molecule; the greater the probability of hydrogen bond formation between the substituent and the stationary phase, then the greater will be the attachment to this phase and the lower will be the R_F of the molecule.

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NOTE—References 1 and 2 are to Parts I and II, respectively, of this series.

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Statistical Analysis of Spectrophotometric Determinations of Boron

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One of the methods for determining boron is by absorption spectrophotometry of solutions of the red compound formed by boric acid and curcumin. The course of this reaction is greatly affected by various factors, and results are sometimes erratic. Those factors causing errors can be detected by not too elaborate methods for interpreting results; other factors, however, cannot be easily interpreted as the probable cause of inconsistency between determinations. This paper describes the application of modern statistical methods to the study of the influence of certain factors on quantitative determinations of boron. The application of these methods provided objective ways of establishing significant effects for the various factors involved.

THE red compound formed when curcumin reacts with boric acid in the presence of oxalic acid has been used in the determination of boron,[1,2,3] and trichloroacetic acid has been used instead of oxalic acid to intensify the colour.[4] However, as observed by Spicer and Strickland,[2] the poor precision generally reported for the method has prevented the widespread use of curcumin for determining boron.

During determinations of the boron contents of graphites in this laboratory, it was observed that results were inconsistent and that there was a general lack of reproducibility between determinations on the same sample unless the various operations were subjected to a degree of control not generally adopted in ordinary analytical procedures. Small variations in the drying time of the red compound, in the amount of moisture in the atmosphere, in the rate of evaporation, in the water content of the ethanol used and in the temperature of drying seemed to cause widely scattered results. Chirnside, Cluley and Proffitt[5] have called attention to the fact that the colour produced by this reaction is influenced by temperature, time of heating and proportion and manner of addition of reagents. Spicer and Strickland[2] recommended that the amount of water in the ethanol used should not be more than 25 nor less than 5 per cent. by volume and that high reproducibility of atmospheric moisture conditions should be maintained. Albinatti and Pazqués[3] observed that lack of simultaneity in drying sample and standard was highly critical, it being absolutely necessary that the evaporations were carried out simultaneously; non-compliance with these conditions gave inconsistent results.

The variations noted by us, however, were not so great that qualitative examination of the results permitted us to ascribe the lack of reproducibility to one or more of the factors mentioned above. A more rigorous examination was necessary, and it was decided to make a statistical analysis of the results to check the level of significance of the differences found. A factorial design[6] was made, in which the factors to be studied were those not already exhaustively examined by Spicer and Strickland,[2] *i.e.*, the volumes of the crucibles and the temperature of drying. In order to study the influence of the latter factor, it was necessary to use at least two temperatures for drying. The levels chosen were 100° C (the temperature normally used[4]) and 55° C (the temperature chosen by Borrowdale, Jenkins and Shanahan, who used oxalic acid to intensify the colour[7]). This choice of temperatures might seem unrealistic, since drying takes about 1 hour at 100° C and 8 hours at 55° C; however, these values were chosen so that the effect of temperature would be detectable and would permit definite statistical interpretation.

This paper describes the statistical aspects of the problem of ascertaining whether the results found were truly different or whether the differences were caused by experimental errors. The importance of design and statistical interpretation in the detection and correction of sources of errors in analytical procedures has been previously emphasised.[8,9,10,11]

Experimental

Preliminary experiments were made with boric acid and curcumin, trichloroacetic acid or oxalic acid being used to intensify the colour. It was observed that the influence of small variations in the various operations was much greater with oxalic acid than with trichloroacetic acid. The chemical aspects of the various operations with oxalic acid had been exhaustively examined,[2] and the method involving use of trichloroacetic acid appeared to be less subject to small variations in technique.[4] However, certain factors seemed to affect the results when trichloroacetic acid was used. We found that the volumes of the quartz crucibles in which the boric acid - curcumin mixtures were evaporated were extremely important; results were shown to be greatly influenced by these volumes, optical-density readings being lower when larger crucibles were used. For this reason, the volumes of the crucibles were included in the factorial design.

An interaction of the factors temperature, volumes of crucibles and range of concentrations of boron might occur, and for this reason, as well as to check whether or not Beer's law was obeyed over the range of boron used (2·5 to 7·5 μg), the concentration of boron was included as one of the factors to be studied.

The chief advantage of the trichloroacetic acid - curcumin method is its high sensitivity, and it is likely to be most valuable in determining amounts of boron appreciably less than 1 μg. Although the choice of the range 2·5 to 7·5 μg of boron had been dictated by the boron contents of samples of graphite, it was decided also to investigate amounts of boron less than 1 μg.

During the preliminary experiments it was observed that some boron was lost if the solutions were not made alkaline for the evaporation to dryness preceding colour development. In order to verify the importance of the errors introduced by not making the solutions alkaline, the set of experiments was repeated with 0·33- and 0·66-μg amounts of boron per crucible, and the solutions were made alkaline with sodium hydroxide before being dried. The other factors—temperature and volumes of crucibles—were the same as in the experiments in which the solutions were not made alkaline.

Method

Reagents—

All reagents were of recognised analytical grade.
Boric acid solution—Containing 10 μg of boron per ml.
Trichloroacetic acid solution, 163 *g per litre.*
Curcumin solution, 0·125 *per cent. w/v in ethanol.*
Ethanol, 95 *per cent.*
Sodium hydroxide, 0·1 N.

Procedure—

Different amounts of boric acid solution were placed in quartz crucibles of capacity 25, 30 and 50 ml, and 0·1-ml portions of 0·1 *N* sodium hydroxide were added to those solutions used in the tests to study the effect of alkalinity. The contents of all the crucibles were then slowly evaporated to dryness on a hot-plate. After evaporation, 1 ml each of trichloroacetic acid and curcumin solutions were added to each residue, and the crucibles were placed in an oven at either 55° or 100° C until constant weight was attained.

To determine the time taken to attain constant weight, identical tests were carried out in parallel with the sample tests. These crucibles were removed from the oven, allowed to cool and weighed at intervals until no change in weight was detected; 15 minutes after this time, the crucibles containing the test samples were removed from the oven. This procedure was considered to be better than the periodic withdrawal, cooling and weighing of the sample crucibles, which would give a much greater chance of variable results.

After the crucibles had cooled, the dry residues were dissolved in ethanol, and each solution was made up to 25 ml. For amounts of boron between 2·5 and 7·5 μg, these solutions were too concentrated for spectrophotometric readings and were therefore diluted ten times with ethanol. The solutions were placed in quartz cells (1 cm × 2 cm), and optical-density readings were made at 5500 A and a slit width of 0·10 mm with a Uvispek spectrophotometer. Blank determinations on solutions containing the same amounts of trichloroacetic acid and curcumin were carried out for each experiment.

Design of Experiments

A 2^3 factorial design was chosen, with three factors: volume of crucible, V, amount of boric acid, C, and temperature, T. In order not to make the blocks too large, a confounding of the interaction of the three factors (VCT) was made, with a comparison between blocks (days of experiments); in this way, the size of the blocks was reduced by half. The loss in efficiency in estimating the interaction VCT was not important, since interactions of high order are seldom significant.[9] The drawback of the design was that it was not possible to check Beer's law over the interval of concentration used; the concentration factors should figure in experiments at three levels at least. In order to obtain the required information about Beer's law, a preliminary complete factorial design, $2 \times 3 = 6$, with seven repetitions (forty-two determinations), was made. In this preliminary design, the temperature was kept at 55° C, the capacities of the crucibles at 25 and 30 ml and the amounts of boron at 2·5, 5·0 and 7·6 μg, so covering the range of the analytical work previously mentioned. The distribution of the six combinations VC in each block was randomised by using Fisher and Yates's Tables.[12]

This preliminary design showed that the main effect of V was highly significant and amounted practically to certainty (the level of significance was less than 0·1 per cent.). This shows that the result found would depend on the size of the crucible; the larger crucibles used furnished consistently lower readings. The main effect of C was highly significant (the level of significance was much less than 0·1 per cent.), as would be expected, and so this effect was subdivided into a "linear term" and a "quadratic term," the coefficients of orthogonal polynomials for $n' = 3$ being used (see Fisher and Yates[12]). The analysis showed that only the first term was significant and that consequently, in the range of concentrations studied, Beer's law was obeyed. This conclusion was important, since it permitted the use of factor C at only two levels, provided that the maximum amount of boron in each crucible was 7·5 μg.

The magnitude of the sum of squares "between blocks" strongly suggested that there were significant differences in the readings for nominally identical samples if the determinations were not made on the same day.

The interaction VC was not significant, even at the 10 per cent. level, and volumes of crucibles and amounts of boron can be considered practically independent.

The information from the preliminary experiments suggested a factorial design of four pairs of blocks (days) with four observations in each block (a total of thirty-two determinations). In relation to a design without confounding, the efficiency of estimating the main effects and the interactions of the factors taken two by two was improved rather than diminished. The analysis of variance corresponding to the chosen design is shown in Table I.

Table I

Analysis of variance for the designed experiments

Source of variation	Degrees of freedom	
Between pairs of blocks (days)	3	
Interaction VCT	1	
Error (residual variance between blocks, by difference)	3	
Between blocks		7
Main effect of V	1	
Main effect of C	1	
Main effect of T	1	
Interaction VC	1	
Interaction VT	1	
Interaction CT	1	
Error (within blocks, by difference)	18	
Within blocks		24
Total		31

The set of determinations to be made on each of the two days in a given pair of blocks was selected at random, and by mere chance the sets chosen for the first days in the four pairs of days were identical. The order of determinations in each day, imposed by the adopted technique of confounding, was also distributed at random. The contents of the blocks, as obtained, for the experiment in which the solutions were not made alkaline before evaporation are shown in Table II, and the optical densities found in this experiment are shown in the

same order in Table III. In Table II, vct is the combination $V_1C_1T_1$, t is the combination $V_0C_0T_1$, v is the combination $V_1C_0T_0$, vc is the combination $V_1C_1T_0$, (1) is the combination $V_0C_0T_0$ and so on.

Table II

Design followed for spectrophotometric determinations

First pair of blocks		Second pair of blocks		Third pair of blocks		Fourth pair of blocks	
First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day
vct	vc	t	vc	v	vc	vct	vt
t	vt	vct	vt	t	(1)	v	(1)
v	(1)	v	(1)	vct	ct	t	ct
c	ct	c	ct	c	vt	c	vc

Table III

Optical-density values

First pair of blocks		Second pair of blocks		Third pair of blocks		Fourth pair of blocks	
First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day
0·337	0·369	0·190	0·345	0·128	0·330	0·355	0·176
0·197	0·173	0·331	0·171	0·189	0·174	0·160	0·172
0·150	0·189	0·151	0·182	0·345	0·350	0·182	0·360
0·367	0·374	0·365	0·374	0·349	0·132	0·362	0·362

For calculation purposes, the results in Table III were codified by multiplying the values by 1000 and subtracting 200; the analysis of variance is shown in Table IV.

Table IV

Analysis of variance of optical-density values

Source of variation	Degrees of freedom	Sum of squares	Mean square	F†
Between pairs of blocks	3	1830·8	610·3	4·21
Interaction VCT	1	175·8	175·8	1·21
Remainder, by difference	3	435·1	145·0	—
Between blocks (days)	7	2441·7		
Main effect of V	1	4072·5	4072·5	42·34***
Main effect of C	1	273,615·0	273,615·0	2844·53***
Main effect of T	1	205·0	205·0	2·13
Interaction VC	1	357·8	357·8	3·72?
Interaction VT	1	30·0	30·0	(3·21)
Interaction CT	1	504·0	504·0	5·24*
Remainder (error), by difference ..	18	1731·5	96·19	—
Within blocks	24	280,515·8		
Total	31	282,957·5		

† Following the usual notation,[9] three asterisks indicate a level of significance less than 0·1 per cent. and one asterisk indicates a level between 1 and 5 per cent. The question mark corresponds to a level between 5 and 10 per cent. ("possibly significant"[13]). The value shown in parenthesis corresponds to a comparison between 18 and 1 degrees of freedom.

The calculation of the various items in Table IV was made by the method described by Davies.[6] For the experiments in which the solutions were made alkaline before evaporation the design was the same, *i.e.*, 2^3, with confounding of the interaction VCT, in eight blocks (days), giving thirty-two readings.

Discussion of results

The analysis of variance confirms the high significance of the main effects of V established in the preliminary experiments. On the other hand, the results for temperature in Table IV show that its main effect is not significant. Since the number of degrees of freedom of the residual variance, 18, is sufficiently high, it is concluded that the temperature of drying does not affect the results. This means that the time of drying can be shortened by using a high temperature (up to 100° C); further, rigorous control of temperature is useless. This conclusion, however, is made with caution, as the interaction CT is of some significance.

The interaction VC is only "possibly significant." If it is borne in mind that the main effects of factors V and C are highly significant, it is plausible to consider V and C as independent, as shown by the preliminary experiments, at least within the accuracy of this work. Practically, this means that crucibles of any volume can be used, provided that the volume is the same for all samples, blanks and calibration measurements.

The statistical analysis does not show any significant interaction VCT; significance, however, would not be indicated unless the effect were very large.

The comparison between pairs of blocks was also of no significance. When the null-hypothesis of no significance of the differences between blocks was made and the mean square between blocks ($2441{\cdot}7 \div 7 = 348{\cdot}8$) was compared with the residual variance within the blocks (96·19), the value of F was 3·63, which is significant at the 5 per cent. level; the preliminary experiments showed still greater significance.

The significance of the variation between blocks shows that the choice of the "day" to define the blocks was judicious; in this way, it was possible to increase the precision of comparisons appreciably. The results of experiments in which the precaution of choosing the "day" to define the blocks was not taken were erratic, and statistical analysis was practically impossible.

All the experimental work was carried out by the same analyst and in nominally identical circumstances; consequently, the experimental error would be expected to be the same in the preliminary experiments and in the experiments reported in Table III. In fact, by comparing the residual variances of both statistical analyses, the value obtained for F is 1·46 (18 against 30 degrees of freedom; double-tailed test[12]), which is not significant. Instead of calculating the standard deviations independently for the preliminary experiments and for the experiments reported in Table III, therefore, a more precise estimate of the error can be found by combining the two "remainders" from both sets of experiments; the coefficient of variation calculated in this way was 0·034, *i.e.*, the relative error was 3·4 per cent. This value gives a measure of the reproducibility of results and represents the error of determinations made on pure solutions. This error may evidently be larger when the determination is made on ores or other materials.

The choice of relative error instead of standard deviation to characterise the experimental accuracy was made because a larger deviation in absolute value might be expected at higher concentrations. A check for homogeneity of variance was not made, as a certain lack of homogeneity in the experimental field has little effect on the accuracy of analyses of variance, even when the results are far from homogeneous.[13]

The experiments in which the samples were made alkaline before evaporation showed the importance of carrying out the various operations on samples and blanks in parallel, since significant differences between blocks (days) were observed (see Table VII). However, no significance was indicated for the factor "volume," which shows that the greater effects of evaporation with larger crucibles were suppressed by making the solutions alkaline.

For these experiments, the sequence of operations used for the calculation, the optical-density readings and the analysis of variance are shown in Tables V, VI and VII, respectively.

Table V

Design followed for experiment with alkaline solutions

First pair of blocks		Second pair of blocks		Third pair of blocks		Fourth pair of blocks	
First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day
c	(1)	vct	vc	ct	t	(1)	vct
vct	vt	c	ct	vc	v	vc	v
t	vc	v	vt	vt	c	ct	t
v	ct	t	(1)	(1)	vct	vt	c

Table VI

Optical-density values for experiment with alkaline solutions

First pair of blocks		Second pair of blocks		Third pair of blocks		Fourth pair of blocks	
First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day
0·415	0·223	0·408	0·415	0·423	0·231	0·225	0·408
0·407	0·245	0·415	0·425	0·414	0·209	0·413	0·211
0·199	0·414	0·210	0·235	0·223	0·418	0·417	0·229
0·210	0·419	0·212	0·221	0·225	0·408	0·219	0·418

Table VII

Analysis of variance for experiment with alkaline solutions

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Between pairs of blocks	3	22·8	7·6	13·43*
Interaction VCT	1	684·5	684·5	6·70?
Remainder, by difference	3	306·2	102·1	
Between blocks	7	1013·5	144·8	3·10*
Main effect of V	1	136·1	136·1	2·91
Main effect of C	1	302,253·1	302,253·1	6465·31***
Main effect of T	1	84·5	84·5	1·81
Interaction VC	1	112·5	112·5	2·41
Interaction VT	1	120·1	120·1	2·57
Interaction CT	1	136·1	136·1	2·91
	6	302,842·5		
Remainder (error), by difference ..	18	841·5	46·7	
Within blocks	24	303,684·0		
Total	31	304,697·5		

Conclusions

The optical-density readings made for nominally identical samples on different days differ significantly. This difference, shown by the statistical analysis, might be caused by variations in atmospheric moisture.

The size of the crucibles affects the results significantly unless the solutions are made alkaline before evaporation, but this effect is independent of the amount of boron present and of temperature.

Because of these conclusions, only one size of crucible should be used for the determination, which should be carried out at the same time for samples and standards.

The temperature of drying does not affect the results, *i.e.*, higher temperatures can be used, so permitting economy in time; further, rigorous control of temperature is not necessary.

The relative error for each reading is about 3·4 per cent. for the range 2·5 to 7·5 μg of boron (solution not made alkaline before evaporation) and 2·2 per cent. for the range 0·33 to 1 μg of boron (solution made alkaline).

Statistical analysis of the results of the preliminary experiments showed that Beer's law was obeyed for amounts of boron up to 7·5 μg per crucible under the conditions described.

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Recommended Methods of Analysis of Pesticide Residues in Foodstuffs

REPORT BY THE MALATHION PANEL

SET UP JOINTLY BY THE SCIENTIFIC SUB-COMMITTEE OF THE INTER-DEPARTMENTAL ADVISORY COMMITTEE ON POISONOUS SUBSTANCES USED IN AGRICULTURE AND FOOD STORAGE, THE ANALYTICAL METHODS COMMITTEE OF THE SOCIETY FOR ANALYTICAL CHEMISTRY, AND THE ASSOCIATION OF BRITISH MANUFACTURERS OF AGRICULTURAL CHEMICALS

The Determination of Malathion Residues in Cereals and Oilseeds

IN May, 1959, a representative meeting of the Scientific Sub-Committee of the Inter-departmental Advisory Committee on Poisonous Substances Used in Agriculture and Food Storage, the Analytical Methods Committee of the Society for Analytical Chemistry and the Association of British Manufacturers of Agricultural Chemicals agreed to set up a Panel (see Appendix II) to undertake collaborative studies of analytical methods for determining malathion in stored products.

Malathion [S-1:2-di(ethoxycarbonyl)ethyl dimethyl phosphorothiolothionate] is a contact insecticide of use in the control of stored-products pests.[1]

A procedure is described for determining malathion residues in groundnuts, cocoa, flour, barley, wheat, oats and maize. The malathion extracted in carbon tetrachloride is decomposed, by adding ethanol and sodium hydroxide, to sodium dimethyl phosphorothiolothionate, sodium fumarate and ethanol. The sodium dimethyl phosphorothiolothionate is then converted to the cupric salt, which is soluble in carbon tetrachloride with the formation of an intense yellow colour. The colour is measured absorptiometrically at 418 mμ, the absorption maximum. Up to 14 p.p.m. of malathion may be present in cereal grains or oilseeds, and the limit of detection of the method with most cereal products is about 15 μg in a 50-g sample, *i.e.*, 0·3 p.p.m. Interference with this method by the presence of other organo-phosphorus compounds has recently been reported.[2] Collaborative work on this method by different laboratories is also reported. The selected method is described in detail and the results of the collaborative work are given.

Two analytical methods were considered; the first was the subject of a private communication from the American Cyanamid Co., Stamford, Connecticut, U.S.A., and the second was the method described by Norris, Easter, Fuller and Kucher[3] for determining malathion residues in animal products. From an examination of these two procedures the composite method described in Appendix I was approved after collaborative analyses carried out in the various laboratories of the Panel's members.

COLLABORATIVE ANALYSIS

The work of the Panel was aimed at devising a detailed procedure that, when applied throughout the country in laboratories of industrial firms, public analysts or in government

establishments under the varied conditions known to exist, would give accurate and reproducible results on as wide a range of foodstuffs as possible.

To check the method, members received samples of untreated wheat and untreated decorticated groundnuts freshly harvested and flown to the United Kingdom by arrangement with Dr. D. W. Hall of the Colonial Office. To each of these samples was added a known amount of malathion (not revealed to members until after they reported their results). The results obtained are shown in Tables I and II.

TABLE I

EXAMINATION OF GROUNDNUTS TO WHICH KNOWN AMOUNTS OF MALATHION HAD BEEN ADDED

Sample A contained 1 p.p.m. and sample B 10 p.p.m. of malathion

Analyst	Malathion found in— control, p.p.m.	sample A, p.p.m.	sample B, p.p.m.
1	Nil	0·9	9·7
2	Nil	2·1	9·1
3	0·3	1·0	10·0
4	Nil	0·4	6·1
5	0·1	1·1	9·1
6	0·1	0·7	10·9
7	Nil	0·1	6·2
8	Nil	1·5	—*
9	Nil	1·0	9·0

* Broken in transit.

TABLE II

EXAMINATION OF WHEAT TO WHICH KNOWN AMOUNTS OF MALATHION HAD BEEN ADDED

Sample C contained 1 p.p.m. and sample D 5 p.p.m. of malathion

Analyst	Malathion found in— control, p.p.m.	sample C, p.p.m.	sample D, p.p.m.
1	Nil	0·3	2·5
2	Nil	Nil	4·7
3	0·3	0·5	3·0
4	Nil	0·3	1·7
5	0·1	0·8	2·6
6	Nil	0·1	2·8
7	Nil	0·5	3·5
8	Nil	0·4	3·0
9	Nil	0·1	2·0

On examination of these results it was agreed that the figures for groundnuts were reasonably satisfactory, but that further work was necessary on wheat. Panel members then carried out individually some extraction and analysis experiments on wheat. Malathion was added at a level of 4 p.p.m. to samples used by all workers.

Experience then showed that—

(*a*) The purity of the carbon tetrachloride was important for successful analysis.

(*b*) The method should specify a water content not greater than 1 per cent. by volume for the ethanol, corresponding to 74° O.P., I.M.S.

(*c*) Differences in techniques of different analysts affected results. Consistent timing of extraction procedures and times taken to complete the analyses, together with the discarding of "cuffs" between layers when separated ensured reproducible results.

(*d*) Of different extraction techniques examined a Soxhlet method gave the best recovery and least trouble with emulsion formation.

(*e*) Of wheat treated with malathion there was a measurable amount of break-down of the chemical over a period of days.

The probability of quick degradation of malathion meant that the discrepancy in results between members could be attributed to the different periods between the preparation of the

samples and their analysis by the various workers. Members therefore agreed to receive a sample of wheat treated with malathion and analyse it exactly 4 days later, or, if in default, to place it in a refrigerator and examine it as early as possible. The results obtained are shown in Table III.

TABLE III

WHEAT SAMPLES TREATED WITH 4 p.p.m. OF MALATHION AND ANALYSED 4 DAYS LATER

Analyst	Malathion residue on day 4, p.p.m.
1	3·5*
2	3·2
3	3·7
4	3·5
5	2·9
6	3·9†
7	4·3
8	3·4

* Analyst who prepared the samples and found 4 p.p.m. after preparation on day 0.
† Average of four analyses extracted in different ways.

The results in Table III are more consistent than are those in Table II and it appears that the time between sampling and analysis is important.

FACTORS AFFECTING EXTRACTION—

One member (S.F.H.) investigated three different extraction methods.

Separate 200-μg portions of solutions of 95 per cent. commercial malathion in carbon tetrachloride were added to each of a number of 50-g samples of wheat. The carbon tetrachloride was allowed to evaporate and the bottles were closed and then stored at room temperature until required for test. Samples of the treated grain were then extracted by the methods described below.

(*i*) *Mechanical shaking*—A 50-g sample of wheat was shaken in a 250-ml polythene-stoppered glass bottle on a mechanical shaker with 50 ml of carbon tetrachloride for 2 hours. The extract was filtered, and the extraction repeated for a further 2 hours with a fresh 50-ml portion of carbon tetrachloride.

(*ii*) *Ball mill*—A 50-g sample of wheat and 50 ml of carbon tetrachloride together with eight 1-inch porcelain balls were revolved at 80 r.p.m. in a 1-litre porcelain jar on rollers. After 2 hours the solvent was filtered and the extraction continued for a further 2 hours with a fresh 50-ml portion of solvent.

(*iii*) *Soxhlet extraction*—A 50-g sample of wheat was extracted for 4 hours with 200 ml of carbon tetrachloride.

CONTROL TESTS—

(*a*) Untreated wheat (50 g) was extracted by each of the three methods; 197 μg of malathion were added to each extract and, on analysis, nearly 100 per cent. recoveries were obtained.

(*b*) Untreated wheat was extracted by method (*i*) and analysed; no malathion was found.

ADDITION TESTS—

Samples of treated wheat were extracted by method (*i*), (*ii*) or (*iii*) at periods of 19, 26 and 38 days after preparation; recoveries are shown in Table IV.

At the 4 p.p.m. level there was some loss or decomposition of malathion on treated whole wheat. The Soxhlet method of extraction was the best of those examined; 60 per cent. of the malathion added was recovered, even after 38 days' storage. It was noted that milling also increased the tendency to encounter emulsions during the analytical procedure. The constancy of recovery suggested that the deficiency was due to decomposition of the malathion originally present and not to losses during extraction.

DEGRADATION AT DIFFERENT TEMPERATURES—

To check the break-down of malathion in wheat over a period, one of the members (L.D.) carried out the experiment described below.

TABLE IV

MALATHION RECOVERED AFTER EXTRACTION BY THREE DIFFERENT METHODS

Storage time, days	Extraction method	Recovery, %
19	Shaking machine	58
26		48
38		43
19	Ball mill	65
26		57·5
38		60*
19	Soxhlet	67·5
26		61·5
38		61

* Operator inadvertently used 16 instead of 8 balls, thereby obtaining a noticeably increased disintegration of the wheat and a recovery of malathion rather higher than expected.

Some 50-g samples of wheat in 4-oz bottles were dosed with 200 μg of malathion in 2 ml of carbon tetrachloride, *i.e.*, 4 p.p.m., screw caps were fitted after evaporation of the solvent, and the bottles were divided into two sets. One set was stored in a cupboard at room temperature (22° to 26° C) and the other in a refrigerator (0° to 5° C).

Samples were removed at intervals, ground with a pestle and mortar, and extracted with carbon tetrachloride in a Soxhlet apparatus for 4 hours. Malathion in the extracts was determined by the recommended method. Four samples were analysed similarly, omitting the milling process; the results are shown in Table V.

TABLE V

EFFECT OF TEMPERATURE AND STORAGE ON THE BREAK-DOWN OF MALATHION IN WHEAT

Values in brackets refer to unmilled samples

Time of storage	Malathion found after storage at— 22° to 26° C, p.p.m.	0° to 5° C, p.p.m.
0	3·6	3·6
1 day	3·4	3·6
1 week	2·8	3·5
3 weeks	2·2 (1·8)	3·1 (3·1)
6 weeks	1·6 (1·3)	3·3 (3·0)

The results indicate that the milling of wheat improves the extraction efficiency and that losses of malathion are greater at the higher storage temperature.

EFFECT OF THE MOISTURE CONTENT OF WHEAT ON THE RECOVERY OF MALATHION—

The effect of moisture content on the decomposition of malathion in wheat was also investigated (L.D.). The moisture contents of two samples of wheat were adjusted by the methods described below.

(*a*) A 400-g portion of wheat was dried by open exposure on a tray at room temperature for 3 days.

(*b*) A similar portion was spread on a tray to a depth of 2 to 4 mm and sprayed with 30 ml of water as evenly as possible. The samples were then mixed thoroughly and stored in a stoppered flask for 3 days to equilibrate. Excess of moisture was removed by again spreading on a tray and drying at room temperature for 6 hours.

The moisture contents were determined by grinding approximately 20-g portions of the wheat, weighing 5- to 7-g amounts, drying at 120° C for 4 hours, and re-weighing. The moisture contents were found to be 12·3 per cent. for (*a*) and 15·3 per cent. for (*b*).

n-Hexane was used as the vehicle for the malathion in preference to carbon tetrachloride to avoid undesirable side effects due to decomposition of the latter solvent.

Some 50-g portions of the wheat were weighed into 4-oz bottles, 1 ml of a solution containing 200 μg per ml of malathion in *n*-hexane was then added dropwise to each bottle, the

wheat being continuously agitated on a flask shaker, which imparted a circular motion to the grains. Agitation was continued for 5 minutes and the bottles were left for 2 hours to allow the hexane to evaporate; a gentle current of air was applied occasionally to assist in removing the solvent. The bottles were capped, and stored at room temperature (21° to 23° C).

The efficiency of blending was checked by dividing one of the 50-g portions into 2 equal portions, (i) and (ii), and determining the malathion content in the usual way. The bottle was also rinsed thoroughly with carbon tetrachloride, which was then examined for malathion content. The results were—

(i) 91 μg of malathion (3·6 p.p.m.)
(ii) 96 μg of malathion (3·8 p.p.m.)
Rinsings 4 μg of malathion.

One-millilitre portions of the *n*-hexane solution were analysed and were found to contain 212 μg of malathion. It was noted that the pipette used for the additions delivered 1·05 ml of *n*-hexane, which accounts for the larger amount of malathion found, 212 μg per ml, instead of the theoretical 200 μg.

Results—The treated wheat samples were analysed at weekly intervals by crushing, extracting the wheat in a Soxhlet apparatus for 4 hours with carbon tetrachloride and examining the extract in the usual way. The results obtained are shown in Table VI. Values quoted are the results of single determinations. The percentage recovery is expressed on the initial level found, *i.e.*, 3·6 p.p.m.

TABLE VI

RECOVERY OF MALATHION FROM WHEAT STORED AT DIFFERENT MOISTURE LEVELS

Storage interval, weeks	Malathion recovered from wheat of 12·3% moisture content, %	Malathion recovered from wheat of 15·3% moisture content, %
1	88	76
2	77	68
3	82	70
4	71	53
5	60	51

The results provide support for field observations with insects suggesting that the persistence of malathion in wheat decreases with increase in moisture content.[4]

EXAMINATION OF MALATHION RESIDUES IN WHOLE-MEAL WHEAT FLOUR—

Recovery experiments were carried out with flour to check the usefulness of the analytical method with this material (E.Q.L.). Extraction of 25 g of flour was carried out in a Soxhlet apparatus; the recoveries were—

Malathion added, μg ..	51	102	152	203
Recovery, %	94	83	80	85

CONCLUSIONS—

The results show that the method described in Appendix I is satisfactory.

It was noted that, when examining malathion residues in foodstuffs, regard would have to be paid to the fairly rapid initial break-down that may be encountered with some commodities.

One or more individual members have also found the method suitable for determining malathion residues in flour, barley, oats, maize and cocoa, as well as in wheat and groundnuts.

Appendix I

RECOMMENDED METHOD

REAGENTS—

Carbon tetrachloride, B.P.—This may contain phosgene or other decomposition products that seriously interfere in the method. Purification with ammonium or sodium hydroxide and then drying and distillation gives a satisfactory product. Carbon tetrachloride, B.P., as purchased may be satisfactory, but should be checked by running a control test.

Ethanol—Ethanol containing not more than 1 per cent. of water (74° O.P. industrial methylated spirit is suitable).

Carbon disulphide solution—A solution containing 1 ml of carbon disulphide in 200 ml of carbon tetrachloride.

Sodium sulphate solution, 9 *per cent. w/v*—Prepare from analytical-reagent grade anhydrous sodium sulphate.

Hydrochloric acid, sp.gr. 1·18—Analytical-reagent grade.

Hydrochloric acid, 6 N.

Ferric chloride solution—Dissolve 5 g of analytical-reagent grade ferric chloride, $FeCl_3.6H_2O$, in 100 ml of *N* hydrochloric acid.

Copper sulphate solution, 3·5 *per cent. w/v*—Prepare from analytical-reagent grade copper sulphate, $CuSO_4.5H_2O$.

Sodium hydroxide, 6 N.

EXTRACTION PROCEDURE—

For cereal products (raw grains) use approximately 50 g of the grain; for oilseeds (*e.g.*, groundnuts and cocoa) use approximately 25 g of decorticated nuts or beans, which should be placed in a mortar and reduced to fragments approximately $\frac{1}{8}$ or less of the original size of the seed. Transfer the grain or oilseeds to a suitable Soxhlet thimble (wash out the mortar into the top of the Soxhlet after fragmenting oilseeds). Extract for 4 hours with a suitable amount of carbon tetrachloride. Take care to see that local overheating of the extract does not occur at the sides of the flask. Adjust the volume of the extract to 100 ml before analysis.

PROCEDURE FOR DETERMINING MALATHION—

Transfer the 100 ml of extract to a 250-ml separating funnel. Add 1·0 ml of the carbon disulphide solution and 25 ml of ethanol, and mix by gentle swirling. Add 75 ml of sodium sulphate solution acidified with 2·5 ml of hydrochloric acid, sp.gr. 1·18, and shake vigorously for one minute. Filter the carbon tetrachloride layer through a fluted, fast filter-paper into a dry 250-ml separating funnel. Do not allow any of the aqueous layer to run on to the filter-paper, and do not wash the paper.

Add 25 ml of ethanol to the filtered carbon tetrachloride solution, then add 1·0 ml of 6 *N* sodium hydroxide, and shake for exactly one minute. (Reaction periods of less than 30 seconds and more than 2 minutes produce low results.) Carry out the procedure from this point onwards without interruption, as aqueous alkaline and acid solutions of dimethylphosphorodithioic acid are stable for short periods only.

Immediately add 75 ml of sodium sulphate solution, and shake vigorously for one minute. Allow the layers to separate, and discard the carbon tetrachloride layer. Add 25 ml of carbon tetrachloride, shake vigorously for 30 seconds, and discard the carbon tetrachloride layer. Add to the contents of the separating funnel 25 ml of carbon tetrachloride, 2 drops of phenolphthalein indicator solution and 6 *N* hydrochloric acid dropwise, with swirling, until the pink colour disappears; then add 1·0 ml of 5 per cent. ferric chloride solution.

Shake vigorously for 30 seconds, and discard the carbon tetrachloride layer. Repeat the extraction twice more with 25-ml portions of carbon tetrachloride, and discard each portion. Add by pipette 15·0 ml of carbon tetrachloride and 1·0 ml of copper sulphate solution, shake vigorously for one minute, and allow the layers to separate. Run the carbon tetrachloride layer into a small beaker containing about 2 g of anhydrous sodium sulphate. Swirl the beaker for a few seconds, and pour off the carbon tetrachloride solution into a 4-cm absorptiometer cell. Measure the optical density of the solution, within 5 minutes of extraction to avoid fading, at 418 mμ.

The capacity of the 4-cm cells varies depending on the instrument and a different volume of carbon tetrachloride may be required in the final extraction. The sensitivity of the method and the slope of the curve will then vary accordingly.

Prepare a standard curve from commercially pure malathion to cover the range 50 to 250 μg of malathion. The curve is essentially a straight line within this range when a 4-cm cell is used, the slope being approximately 3 optical-density units per mg of malathion.

It should be noted that there are two critical stages in the method. The first during the hydrolysis with 6 *N* sodium hydroxide and the second when the copper sulphate is added. Precise timing and the discarding of "cuffs" between layers helps in obtaining good results. Sodium sulphate may also crystallise out, but this can be overcome by placing the reagent vessels in warm water and gently swirling until the crystals are dissolved.

Emulsions may occasionally be encountered, particularly when working temperatures exceed 25° C or undue grinding and crushing are used before extraction.

Appendix II

MEMBERSHIP OF THE PANEL

The Panel consisted of Angela N. Bates, A. S. Beidas, L. Donegan, E. G. Hill, S. F. Holder, H. Kassler (appointed December, 1959), D. M. Langbridge, E. Q. Laws, C. A. Maunder-Foster (resigned November, 1959), E. J. Miles, D. S. Papworth (Secretary), E. E. Turtle (Chairman) and D. J. Webley.

We thank Cyanamid of Great Britain Ltd. for supplying pure technical malathion and Dr. D. W. Hall for supplying fresh groundnuts from West Africa.

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Notes

THE DETERMINATION OF METHANOL IN BIOLOGICAL FLUIDS

FELDSTEIN and Klendshoj[1] determined methanol in blood and urine by microdiffusion in a Conway cell. This method gives a recovery of about 85 per cent. and a correction is made to allow for incomplete absorption of the methanol by the sulphuric acid. A modification that does not require a correction factor has been devised; a Cavett flask is used.

METHOD

APPARATUS—

Cavett flasks—Obtainable from Quickfit & Quartz Ltd. (catalogue No. 2BC).

Colorimeter—An E.E.L. portable colorimeter (Evans Electroselenium Ltd.), with Ilford No. 626 filter.

Incubator—Temperature controlled at 37° C.

REAGENTS—

Concentrated sulphuric acid.

Sulphuric acid, diluted (1 + 2).

Potassium permanganate solution, 0·3 per cent. w/v.

Sodium sulphite solution, saturated, aqueous.

Potassium carbonate solution, saturated, aqueous.

Chromotropic acid solution—Prepare a 0·5 per cent. w/v solution of the sodium salt of chromotropic acid. This solution must be freshly prepared each day.

Standard methanol solution, 1·0 per cent. w/v—Prepare by diluting 12·5 ml of analytical-reagent grade methanol to 1 litre with water.

Dilute standard methanol solution, 0·05 per cent. w/v—Dilute 5 ml of the 1·0 per cent. solution to 100 ml with water.

PROCEDURE—

In each of three Cavett flasks place, by pipette, 3 ml of diluted sulphuric acid and 1 ml of potassium permanganate solution, and in the cup of each stopper place 0·1 ml of potassium carbonate solution. In one of the cups place 0·5 ml of the blood or urine, and in the others place 0·1 and 0·5 ml of dilute standard methanol solution. Insert the stoppers, and set the flasks aside in an incubator at 37° C for 4 hours. At the end of this period remove the flasks, and allow them to cool to room temperature. Remove the stoppers, and to the contents of each flask add sodium sulphite solution dropwise until the permanganate is decolorised. Add 0·1 ml of chromotropic acid solution and 4 ml of concentrated sulphuric acid. Mix, and suspend the flasks in a bath of

boiling water for 10 minutes. Cool, transfer the contents to 10-ml calibrated flasks, rinse each Cavett flask with two 0·5-ml portions of water, and add the rinsings to the contents of the calibrated flasks. Cool again, and dilute each solution to the mark.

Measure the colour produced at 570 mμ against a blank of the reagents; use an E.E.L. colorimeter, with an Ilford No. 626 filter. The standards correspond to concentrations of 10 and 50 mg of methanol per 100 ml of sample. Beer's law is obeyed up to a concentration of 70 mg per 100 ml. Above this concentration, the colour is too deep to read and the determination should be repeated with a smaller amount of blood or urine.

Results and discussion of the method

Known amounts of methanol were added to samples of blood and urine known to be methanol-free; the recovery results were—

Methanol added, mg per 100 ml of sample	10	20	40	60	65
Methanol found in blood, mg per 100 ml	12	21	41	60	65
Methanol found in urine, mg per 100 ml	11	22	41	60	67

Ethanol does not interfere with the determination. The sulphuric acid solution is presumably sufficiently concentrated to polymerise the formaldehyde produced by oxidation of the methanol by the permanganate, and the vapour pressure of the methanol is reduced to zero. No correction is necessary for incomplete diffusion.

Formaldehyde, if present in the original sample, reacts with the chromotropic acid and enhances the colour. If its presence is suspected, a replicate determination should be carried out without the potassium permanganate. Any colour produced is then due to formaldehyde alone, and the true amount of methanol can be found by difference.

The reaction is complete in 3 hours, but an incubation period of 4 hours is convenient if an ethanol determination by Cavett's method, as described by Kent-Jones and Taylor,[2] is being performed at the same time on another sample.

I thank the Director of Medical Services, Northern Rhodesia, for permission to publish this Note.

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THE DETERMINATION OF TOTAL P_2O_5 AND TOTAL B_2O_3 IN DETERGENT POWDERS

When analysing detergent powders containing sodium phosphates and sodium perborate, it is often desirable to determine the B_2O_3 as well as the available oxygen. The usual gravimetric methods for phosphate separate the two ions, but the subsequent determination of borate in the filtrates is tedious. In the volumetric method described below for the co-determination of phosphate and borate this difficulty has been overcome.

Method

Reagents—

Nitric acid, 3 N.
Sodium chloride solution, saturated.
Silver nitrate solution, 10 *per cent.*
Sodium hydroxide, 3 N.
Sodium hydroxide, N—Accurately standardised.
Sodium hydroxide, 0·1 N—Accurately standardised.
Glycerine.

Procedure—

Weigh 4 to 5 g of detergent powder in a platinum dish. Heat in a bunsen flame until completely charred, transfer to a muffle furnace maintained at 700° to 800° C, and ignite to a white ash. Cool, add a few millilitres of 3 *N* nitric acid, and warm to dissolve the residue. Transfer quantitatively to a boiling flask, using a total volume of nitric acid not less than 25 ml. Attach a reflux

condenser to the flask, and boil for at least 30 minutes. Cool, and transfer the solution to a 400-ml beaker. Insert the glass and calomel electrodes of a pH meter and an electric stirrer. Adjust the pH to between 3·5 and 4 with 3 *N* sodium hydroxide and then accurately to pH 4·5 with *N* sodium hydroxide. Add 100 ml of 10 per cent. silver nitrate solution, and titrate with *N* sodium hydroxide, added from a 50-ml burette fitted with a curved jet, to pH 6·0.

1 ml of *N* sodium hydroxide ≡ 0·0355 g of P_2O_5.

Remove and rinse the electrodes and stirrer. By suction, separate the suspended silver phosphate on two thicknesses of Whatman No. 42 filter-paper. Rinse the beaker with a few millilitres of water, and pour the rinsings through the filter. Add to the filtrate 5 ml of 3 *N* nitric acid and sufficient saturated sodium chloride solution to precipitate all the excess of silver. Agitate until the precipitate has coagulated, filter by suction, and transfer the filtrate to a beaker. Insert the electrodes of the pH meter and the electric stirrer. Adjust the pH to 5·5; use *N* sodium hydroxide for approximate adjustment and 0·1 *N* sodium hydroxide for the final adjustment. Add a volume of glycerine equal to about half that of the solution in the beaker, and titrate slowly to pH 8·5 with 0·1 *N* sodium hydroxide.

1 ml of 0·1 *N* sodium hydroxide ≡ 0·003484 g of B_2O_3.

Results

The proposed method was tested with prepared mixtures of disodium hydrogen orthophosphate and sodium metaborate; the results are shown in Table I.

Table I

Results for prepared mixtures of phosphate and borate

For the mixtures containing *M* borate, the filtrate from the phosphate titration was diluted to 250 ml and the borate was determined in a 25-ml aliquot of this solution

Mixture No.*	Theoretical titre for phosphate, ml	Titre found for phosphate, ml	Theoretical titre for borate, ml of 0·1 *N* sodium hydroxide	Titre found for borate, ml of 0·1 *N* sodium hydroxide
Phosphate titrated with N *sodium hydroxide*—				
1	10·01	10·06	10·10	10·20
	10·01	10·08	10·10	10·15
	10·01	10·10	10·10	10·20
2	22·18	22·18	25·25	25·10
	22·18	22·17	25·25	25·22
	22·18	22·18	25·25	25·20
3	10·01	10·02	10·26	10·18
	10·01	10·02	10·26	10·22
	10·01	10·04	10·26	10·20
4	25·02	25·08	25·65	25·07
	25·02	25·09	25·65	25·15
	25·02	25·07	25·65	25·24
Phosphate titrated with 0·1 N *sodium hydroxide*—				
5	10·00	9·96	10·26	10·20
	10·00	9·98	10·26	10·15
	10·00	9·96	10·26	10·22
6	25·00	24·98	25·65	25·32
	25·00	25·00	25·65	25·30
	25·00	25·00	25·65	25·30

* The mixtures used were—

1. 10 ml each of 0·5 *M* disodium hydrogen orthophosphate and *M* sodium metaborate.
2. 25 ml each of 0·5 *M* disodium hydrogen orthophosphate and *M* sodium metaborate.
3. 10 ml each of 0·5 *M* disodium hydrogen orthophosphate and 0·1 *M* sodium metaborate.
4. 25 ml each of 0·5 *M* disodium hydrogen orthophosphate and 0·1 *M* sodium metaborate.
5. 10 ml each of 0·05 *M* disodium hydrogen orthophosphate and 0·1 *M* sodium metaborate.
6. 25 ml each of 0·05 *M* disodium hydrogen orthophosphate and 0·1 *M* sodium metaborate.

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DETERMINATION OF OO-DIMETHYL S-(N-METHYLCARBAMOYLMETHYL) PHOSPHOROTHIOLOTHIONATE IN TECHNICAL ROGOR AND ITS FORMULATIONS

In our paper[1] on the partition-chromatographic determination of dimethoate, we stated that OO-dimethyl phosphorodithioic acid preceded dimethoate when eluted from the column with a mixture of *iso*propyl ether and light petroleum. The sample of acid used in determining qualitatively the behaviour of this impurity was taken from a batch of technical material.

Subsequent work has shown our original statement to be incorrect. A sample of pure OO-dimethyl phosphorodithioic acid was prepared by one of our colleagues, Mr. D. W. J. Lane. This sample had the correct equivalent weight and elemental analysis. Partition chromatography according to the method originally described showed that no significant acidic or phosphorus-containing material was eluted between 0 and 500 ml.

It was concluded that the original sample tested was grossly impure and that the peak, formerly attributed to OO-dimethyl phosphorodithioic acid, contained an impurity—probably OOS-trimethyl phosphorodithioate.

OO-Dimethyl phosphorodithioic acid is not likely to occur in technical Rogor in large amounts and any traces present will be eluted so long after dimethoate that they will interfere neither with the original determination nor with subsequent ones.

REFERENCE

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A RAPID METHOD FOR DISSOLVING HIGHLY PURE ALUMINIUM FOR ANALYTICAL PURPOSES

THE dissolution of highly pure aluminium in acid is generally difficult, because the methods usually applied effect very slow solution. The use of cupric chloride and hydrogen peroxide in combination with hydrochloric acid, however, permits rapid dissolution of pure aluminium.

Tests in our laboratory showed that a 1-g sample of aluminium having a purity of 99·9 per cent. could be completely dissolved within 5 minutes, without heating, by using a solution consisting of 10 ml each of 36 per cent. hydrochloric acid and 30 per cent. hydrogen peroxide and 1 to 5 drops of a 10 per cent. w/v aqueous solution of analytical-reagent grade cupric chloride. If the solution is heated, the hydrogen peroxide decomposes and the copper is precipitated, thereby losing its activity; it is therefore better to add the hydrogen peroxide in portions during the dissolution. Results were not good when nitric or sulphuric acid was used instead of hydrochloric acid.

When the addition of copper is undesirable, *e.g.*, because it interferes in determinations such as those of copper or iron, the dissolution of samples of pure and highly pure aluminium in hydrochloric acid alone is very rapid when the material is in contact with an electropositive element, for example, platinum or gold. When a platinum crucible was used as container, the aluminium dissolved rapidly in 10 ml of concentrated hydrochloric acid when a few drops of hydrogen peroxide were added as depolariser.

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Book Reviews

THE ANALYSIS OF TITANIUM AND ITS ALLOYS. Third Edition. Pp. 119. London: Imperial Chemical Industries Ltd. 1959. Price 21s.

The appearance of a third edition of this book less than 3 years after the publication of the first shows that there must have been a wide demand for the information that it provides and that the advances in this field of chemical analysis are being made promptly available to the analytical public.

With a few exceptions, the procedures described apply to the examination of titanium and its alloys in cast or wrought form, but, provided that the recommended sampling methods are followed, they apply equally well to samples of titanium sponge or granules.

Methods for twenty-six different elements, as well as for moisture, are detailed, and this edition contains a short section in which the differentiation of titanium and its alloys by means of simple drop-reaction tests is described. A procedure for determining boron, a more sensitive method for nitrogen, a new one for chlorine, an additional method for carbon and a differential absorptiometric method for determining alloying amounts of molybdenum are also innovations.

All the methods recommended are those in use in the Metals Division of Imperial Chemical Industries Ltd. and have either been developed or had their trustworthiness confirmed in the research department of this Division. This inspires confidence in the procedures described and contributes much to the value of the book.

Only one misprint, *viz.*, "additon" for "addition," p. 113, has been noticed. A private communication from the author points out that under the heading "Silver Nitrate Solution $N/100$" (p. 32), the instruction should read "Dissolve 1·699 g.* of silver nitrate in about 250 ml. of water and dilute to 1 litre." The atomic weights listed near the end of the book are out-of-date, and the 1957 values (*J. Chem. Soc.*, 1957, 5101) should take their place.

Before a new edition is prepared, those responsible for it might like to consider the fact that if dilute solutions of potassium permanganate are prepared by boiling and are then filtered from hydrated oxides of manganese, they can be standardised as soon as they are cool and a waiting time (p. 107) is no longer necessary. The conversion of calcium oxalate to the carbonate by heating at 500° ± 20° C could also be adopted. This has much to commend it and is preferable to ignition of the oxalate to oxide before weighing (*cf.* p. 22). The solubility of the nickel dimethylglyoxime complex in hot solutions, which is by no means negligible in accurate work, should be taken care of by cooling before filtration (*cf.* p. 63). Finally, it seems likely that the EDTA procedure described for aluminium (p. 17) can be simplified by a method in which xylenol orange is the indicator, hexamine the buffering agent and standard lead or zinc solution the titrant.

In recent years, some of the larger chemical organisations in this country have published books in which the analytical methods sponsored by their research departments and used in the company laboratories are made available to a wider public. Being accounts of well-tried methods in frequent use, these books are of considerable value to busy analysts by giving them guidance in what is often unfamiliar territory, and analysts should be grateful for a policy that makes such help available to them. This most useful book is one of these, and it should be among the first to be consulted when an analysis of titanium or its alloys has to be undertaken. L. S. THEOBALD

METHODS OF BIOCHEMICAL ANALYSIS. Volume VIII. Edited by DAVID GLICK. Pp. x + 400. New York and London: Interscience Publishers Inc. 1960. Price $10.00; 75s.

This volume opens with the "qualitative and quantitative determination of fatty acids by gas - liquid chromatography," by A. T. James, of London. The article (61 pages) covers nomenclature, principles and techniques. The results of quantitative separations lead to a comparison of the gas chromatogram and the alkali-isomerisation technique for the determination of polyethylenic acids. The two methods agree surprisingly well considering that alkali isomerisation determines acid *types*, *i.e.*, di-ene, tri-ene or tetra-ene acids, whereas gas chromatography is in essence concerned with individual acids. James points out that studies of the biosynthesis of fatty acids are facilitated by the use of gas - liquid chromatography to separate small amounts of the individual acids, which may of course be given a radioactive "label."

A. M. Chase, of Princeton, contributes an article on the measurement of luciferin and luciferase. Three bioluminescent systems, those of luminous bacteria, the fire-fly and a crustacean, *Cypridina hilgendorfii*, have been studied in detail. In each, a fairly heat-stable luciferin is produced, which is acted upon by an enzyme, luciferase, to produce light. The various luciferins (and probably the luciferases) are chemically distinct; cypridina luciferase (molecular weight about 70,000) is apparently a simple protein without a detachable coenzyme grouping, and cypridina luciferin (crystalline; molecular weight 470; $C_{21}H_{28}O_2N_6.2HCl$) has not yet been characterised. Fire-fly luciferin is more stable to oxygen and gives rise to luminescence only when ATP and Mg^{2+}, as well as oxygen, water and luciferase, are present. Bacterial luminescence requires flav mononucleotide and a long-chain fatty aldehyde, as well as dissolved oxygen and bacterial luciferase. Chase

* Incorrect in only a few copies.

gives details for preparing luciferin and luciferase from dried Japanese *Cypridinae* kept in sealed containers with a desiccating agent. The absorption spectra exhibited by crystalline cypridine luciferin in buffer solutions change with time and the curves show good isosbestic points. The absorption curves for crystalline fire-fly luciferin again show that the luciferins are chemically distinct. Technical methods for measuring the light emission are given and assay procedures are described. The requirement of ATP for fire-fly luminescence *in vitro* has led to a very sensitive assay method for adenosine triphosphate (Strehler and McElroy), and a less direct but trustworthy assay method for coenzyme A has also been worked out. Apparently, very low concentrations of oxygen can also be determined.

P. Talalay, of Chicago, writes on enzymic analysis of steroid hormones. Hydroxysteroids can be converted to ketones by certain dehydrogenases in accordance with the reversible reaction—

$$\text{Steroid alcohol} + \text{DPN}^+ \rightleftharpoons \text{Steroid ketone} + \text{DPNH} + \text{H}^+$$

and the absorption peak at 340 mμ ($\epsilon = 6220$) of DPNH is used to follow the process. The occurrence of the appropriate DPN-specific hydroxysteroid dehydrogenases is discussed, and the results are applied to analytical problems and to enzymic resolution of racemic steroids, as well as to the analysis of urinary steroid metabolites. Detailed experimental instructions are given and enzymic and chemical methods are compared.

J. E. Scott, of London, discusses aliphatic ammonium salts in the assay of acidic polysaccharides from tissues. These include hyaluronic acid, chondroitin, chondroitin sulphates (A, B and C) and heparin. Quaternary ammonium detergents (QN^+), *e.g.*, cetyltrimethylammonium bromide or cetylpyridinium chloride, form insoluble salts with polyacids or QN^+ - polyanion coacervates of very low solubility and fixed chemical constitution. Detailed quantitative applications are given and the recovery of polysaccharides from the QN^+ complexes is described.

M. W. Whitehouse and F. Zilliken, of Philadelphia, contribute a concise article on the isolation and determination of neuraminic (sialic) acids. The sialic acids, of which five are known, are important components of mucoproteins and mucolipids and are defined as aminocarboxyoctuloses. They may act as bridges between polysaccharides and polypeptides. The properties of the neuraminic acids are described, with methods for their detection by paper chromatography or ionophoresis and their determination by colorimetric procedures.

Four authors from Minneapolis, led by S. Schwartz, discuss the determination of porphyrins in biological materials. They summarise the chemical properties of porphyrins, their absorption spectra in the visible, ultra-violet and infra-red regions and their fluorescence spectra. The analysis of urine, faeces and tissues for porphyrins is discussed fully, and normal and abnormal values for man and for laboratory animals are given.

A. B. Lerner and M. Ruth Wright, of Yale, describe *in vitro* frog skin assays for agents that darken and lighten melanocytes. The melanocyte-stimulating hormone (MSH) is a darkening factor from the pituitary gland and melatonin is a lightening factor from nerve tissue. Both can be detected at a concentration of about 10^{-12} *M* and assayed with an error of less than 10 per cent. The melanocytes from frog skin are also darkened by ACTH, caffeine and marsilid. Lightening is effected also by adrenaline, noradrenaline and other substances. α-MSH has 13 amino acids arranged in the same sequence as the first 13 amino acids in ACTH; β-MSH has 18 to 22 amino acids in peptide linkage. Detailed assay procedures are given.

C. Kitzinger and T. H. Benzinger, of Bethesda, discuss the principle and method of heatburst microcalorimetry and the determination of changes in free energy, enthalpy and entropy (ΔF, ΔH and ΔS). The original argument (J. M. Sturtevant, *J. Phys. Chem.*, 1941, **45**, 127) was developed by Benzinger in 1956 so that ΔF, ΔH and ΔS are obtained from two measurements of heat, Q_1 with reactants and Q_2 with products, taken during their transformation into the species present at chemical equilibrium—

$$A + B \rightleftharpoons C + D;\ k = Q_1^2/Q_2^2;\ \Delta F^0 = RT \log (Q_1/Q_2)^2.$$

In classical calorimetry a temperature difference is measured between the reactant solution and a blank or heat-sink, but the potential thermal energy of the system is not tapped. In heatburst microcalorimetry, the potential thermal energy is utilised for the measurement. "The heatburst principle demands discharging the heat, at maximal velocity from every segment of the material into the heat-sink through a thermopile in order to convert the energy of thermal flux into a short but powerful signal." The designing of a heatburst microcalorimeter and of reaction cells, together with sound calibration, represents a marked achievement.

Biochemical applications are given: (*a*) ΔH for the hydrolysis by asparaginase of asparagine to ammonium aspartate is -5710 calories per mole and (*b*) ΔH for the enzymic hydrolysis of adenosine

triphosphate by myosin, including the heat of neutralisation of 1 mole of protons (Tris buffer) is −16,360 calories per mole of ATP. The heat of neutralisation was −11,580 calories per mole, making −4800 for the heat of ATP hydrolysis proper. In phosphate buffer, ΔH for the combined reaction is 6815 and for the neutralisation 1835, leaving 4980 calories per mole for the ATP hydrolysis. It appears that in living cells, when ADP and ATP are mostly present as metal (*e.g.*, magnesium) complexes, the value of ΔF^{1}_{310} is −7000 calories per mole. Without magnesium, the free energy would be 8600 calories per mole at pH 7·0. This chapter describes important work with considerable implications.

This volume can be approached in two distinct ways. At one level it is concerned with methods, tools and skills, but at another level it deals with basic ideas and theoretical advances. By any test the work reaches a high standard.

R. A. MORTON

APLICACIONES ANALÍTICAS DEL AEDT Y SIMILARES (Analytical Applications of EDTA and its Analogues). Prof. Dr. FRANCISCO BERMEJO MARTINEZ and Dr. ANTONIO PRIETO BOUZA. Pp. xii + 629. Santiago de Compostela: Imprenta del Seminario Conciliar. 1960. Price $10.00.

The publisher's note states that this aims at being both a text-book and a work of reference for "all those who need to use EDTA and its analogues in analytical chemistry." A brief résumé will indicate its wide scope.

The first half consists of a general discussion of the properties of complexones and of the principles involved in their use; it is divided into chapters as follows. General properties (120 pp.)—this includes the underlying physical chemistry and a discussion of the stability constants of metallic chelates; Uses in qualitative analysis (23 pp.); Chelometric titrations (120 pp.); Gravimetric determinations and analytical separations (30 pp.); Absorptiometric determinations (15 pp.) and Polarographic applications (9 pp.).

The remaining 300 pages contain detailed directions for carrying out some 500 analytical procedures and a very extensive bibliography of 1278 references (many as recent as 1959) from international sources, including a number of the less accessible journals of Eastern Europe, from all of which references have been quoted in the text.

As an introduction to the subject, the book suffers to some extent from its comprehensiveness, and the newcomer to the field might perhaps wish for something more concise; as a reference book, however, it offers much that is of interest. For those anxious to make a rapid survey of the field, if they can read Spanish, it should save much literature searching.

A fuller index, with more cross-references, would have permitted the reader to find his way about more easily, and it is a pity that the bibliography is neither classified nor provided with an author index. References to the determinations of individual substances are, however, satisfactorily indexed. The authors, themselves original workers in the field, are to be congratulated on the compilation of a valuable addition to the literature of these versatile reagents.

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Publications Received

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Complete proceedings of the Colloquium held in September, 1959, in Lucerne.

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A new journal; published by the Forensic Science Society, c/o Rossett Holt, Pannal Ash Road, Harrogate, Yorkshire.

MAGNETIC MATERIALS. By F. BRAILSFORD, Ph.D., Wh.Sch., M.I.E.E. Pp. viii + 188. London: Methuen & Co. Ltd.; New York: John Wiley & Sons Inc. 1960. Price 16s.; $1.50.

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Erratum

SEPTEMBER, 1960, ISSUE, p. 632, Table II. An incorrect factor was used for converting As_2O_3 to As. The Table should read—

Sample 1: procaine hydrochloride with 5 p.p.m. of added As_2O_3 (≡ 3·8 p.p.m. of As)
Sample 2: Antrycide
Sample 3: tobacco powder

Laboratory	Arsenic (As) found in— Sample 1, p.p.m.	Sample 2, p.p.m.	Sample 3, p.p.m.
A	3·4	0·08	—
B	3·7	0·0	—
	3·6		
C	3·4	0·91	2·1
D	3·4	0·11	1·1
	3·4	0·08	